

nature

September 25, 1975

Why industry and academics go their separate ways

ACADEMIC engineers say that industry

- has not the imagination to see long term potential;
- is unreliable when it comes to financial, and even moral, obligations;
- shows little interest even in projects it has backed itself;
- reacts to proposals through its accountants;
- wastes highly qualified manpower;
- is too secretive about its policies and the results of research projects.

For good measure, industry says that academics

- cannot keep to deadlines;
- get distracted from central objectives of projects;
- have too many other responsibilities to manage a project properly;
- are not keen to work to specific contract with a time scale;
- are insensitive to the realities of the market place;
- promise more than they can provide;
- have little notion of the human and environmental contexts of technology.

The caricatures described above do not come from a confidential telephone number to which malcontents could express their anonymous gripes; rather they come from a Science Research Council report published last week and entitled *Academic-Industrial Collaboration in Engineering Research* (SRC, free of charge). We hasten to add that the SRC is not urging these opinions on the community—it is simply reporting, through a panel headed by Professor E. J. Richards (Loughborough University), the common stereotyped views that still circulate widely and undoubtedly have some substance.

The panel, like many predecessors, is concerned at the “far from satisfactory” links between university and industry, and particularly links which develop, or fail to develop, when universities look to the SRC for support in engineering research ventures which are too speculative and risky for industry to pursue in its own laboratories. The problem is put more simply and chauvinistically by politicians and newspapers—“Why do we have so many good ideas and then waste them or let a foreign company exploit them?”

This popular viewpoint may be quite wrong. British companies may steer clear of certain ideas with justification. The panel acknowledges that in some industries (computers, for instance) the collaboration is much easier to bring about. And in some cases the international nature of corporations makes simple-minded talk about British universities benefitting British industry dated and meaningless. But there is no denying that there is still much uneasiness that universities and industry go their separate, mutually incomprehending ways far too much.

The panel sees three serious gaps in the present framework for research support:

- A pre-development gap between researcher and industrialist concerning the time at which a research project can be seen to have an outcome. The academic scents success for an idea long before an industrialist is convinced; the panel urges that bridging work be given much greater support, by either the SRC, the universities, industry or research associations. This is the central message for SRC who have traditionally only supported research.
- An evaluation gap between researchers and industrialists in the following-through of research results. The panel points an accusing finger at the SRC whose record is “very poor” in the dissemination of information so that industry can get early warning of developments.
- An identification gap; it seems that academics would positively welcome a more interventionist approach by the SRC in policy formation and implementation. There is much, says the panel, that the Engineering Board (of the SRC) could do to improve its own ability to identify research needs.

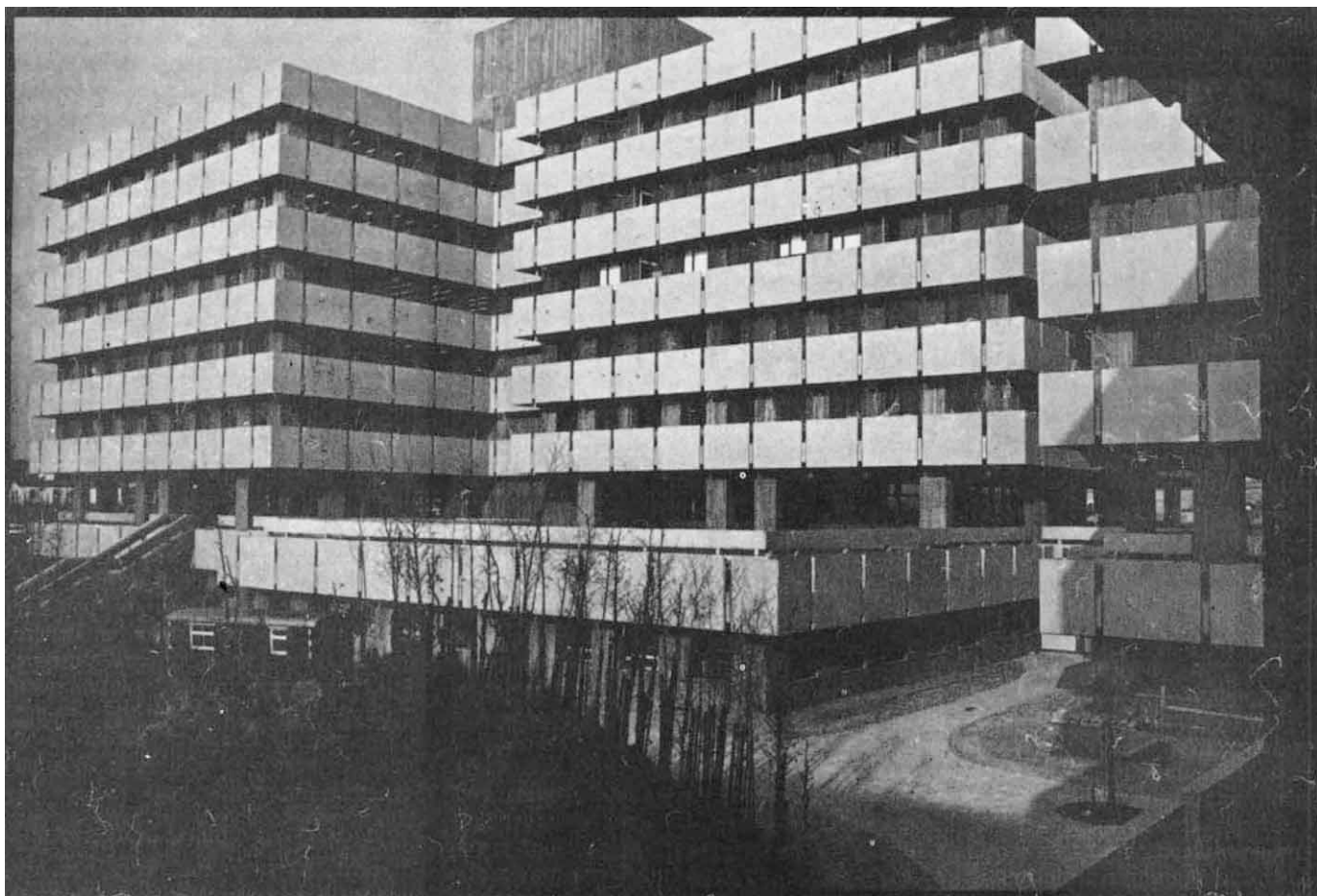
It is striking how much of what the panel says boils down to one problem—adequate communications. Perhaps the most telling section of the report runs: “... success [in collaboration] depends strongly on the compatibility of the individuals in contact, and their enthusiasm for the project. Clearly defined objectives and responsibilities are also important, but rank second to satisfactory human relationships”.

The panel was in no position to do more than make recommendations relevant to the SRC's policy, but it seems abundantly clear that at some time two broader issues will have to be faced.

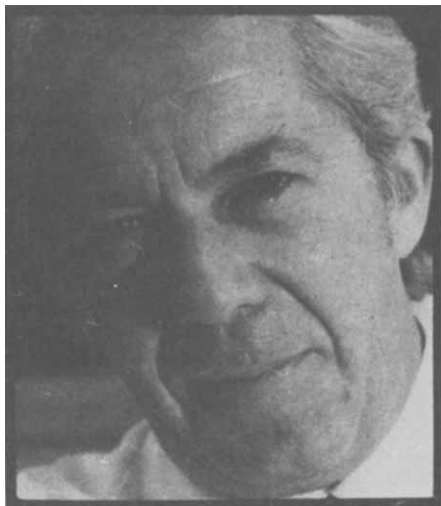
First, are not engineering departments in many universities too isolated? The separation of university entrants into mathematicians, scientists, and engineers, each pursuing separate educational paths (and sometimes attending almost identical lectures) not only makes it more difficult for the student to sample widely and choose appropriately but also encourages an artificial erection of barriers around engineering which is bound to lower the general ability to communicate.

Second, the amount of movement in mid-career between university and industry, either for a year or for an indefinite period, is still pitifully small. It is certainly possible to point to short term exchanges, but if communication is to be improved it must be on the basis of understanding the constraints under which the ‘other side’ works. This can hardly be done in a few weeks, but rather requires people to stay long enough not just to be involved in the making of decisions, but also to have to live with the consequences of those decisions. □





The International Institute of Cellular and Molecular Pathology (above) was inaugurated as a centre for medical research last April. Christian de Duve (below) is president of its governing body as well as chairman of the executive committee and in those capacities he directs the activities of the ICP. The institute is already acting as a honey-pot for expatriate Belgian scientists but it is de Duve's problem to try and make the institute live up to its 'International' intent. Peter Newmark reports from Brussels.



THE ICP will probably be international before it is truly national, having been born out of the long standing Belgian failure to integrate the French and Flemish-speaking communities. Separatist movements at the Université Catholique de Louvain (UCL) triggered off such disruptions in 1968 that co-existence on one campus became impossible. It was during the ensuing upheaval that the idea of the ICP arose, along with a government plan to found a separate French-speaking UCL on Walloon territory. The outcome of subsequent deliberations was a brand new campus in a Brussels suburb. On this campus are sited a 900-bed hospital, the new UCL medical school and the ICP.

In spite of the very close links between the ICP and the medical school, including the fact that all the senior members of the former hold chairs at the UCL, the institute aspires to autonomy, and is well on the way to it. Thus it is registered as an independent body, has its own charter and governing body and maintains a Scientific Advisory Board of international notables who are consulted before any important appointments are made. (Advice in the negative from them has already been followed on occasion.) In addition the official language of the ICP is English.

All research at the ICP is intended to be medically orientated but with

some categorisation into basic and applied projects. The organisation is described, perhaps more for the benefit of laymen and fund-raising than for experienced scientists, as consisting of a central core of basic scientists on to which are attached groups that are tackling more applied problems—the promised advantage being that many diverse groups can obtain adequate basic support from the multiple skills of the core.

So far the majority of the staff, at present numbering about 150 including supporting personnel, belong to the 'central core'. They divide into four main groups. Largest by far is de Duve's biochemistry group which concentrates its work on subcellular particles and the metabolism of both glycogen (under H. G. Hers) and connective tissue (under G. Vaes). The experimental medicine group is the next largest. Led by J. F. Heremans, it provides the immunological know-how of the ICP and has an active interest in various proteins of body fluids. Third, there is a general pathology group operating in the field of endocrinology, particularly thyroid hormones, under M. de Visscher. And finally there is C. Cocito's small microbiology and molecular genetics group. Each of these four groups was located separately until the ICP united them and each is very enthusiastic about its new brotherhood.

But what of the new groups that de Duve is hoping to graft in to, or preferably on to, the existing 'central core'? So far a large grant from a French drug company has enabled A. Trouet to set up an experimental chemotherapy unit, T. Boon is about to join the institute to start up a cellular genetics group that will concentrate on teratocarcinomas and there are advanced plans for a parasitology group backed by the WHO.

Noticeable at this stage largely for their absence are any new clinically orientated research groups. Since these are part not only of the design but also of the justification of the ICP, they will have to be started in due course. The main question that concerns de Duve at present is whether he can find suitable staff to originate and run new groups.

In his favour are the fine facilities of the ICP, the permanent posts that can be offered and the fact that de Duve (no hand-waggoner himself) is prepared to back almost any line of medical research provided he finds the right person. Against him is the considerable problem of attracting the foreign candidates that de Duve is after.

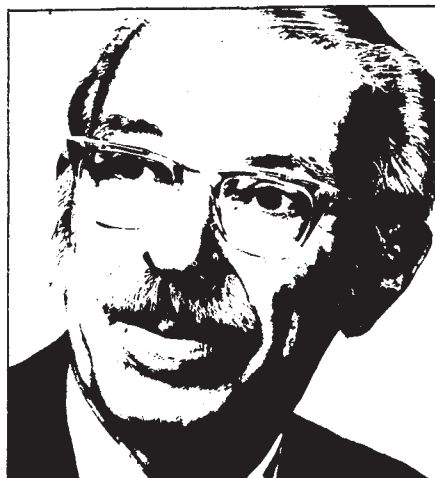
Why look for foreigners? In the first place there simply are not enough top-class Belgian scientists: second, there is the 'International' label to live up to. But over and above those reasons is de Duve's profound belief in the value of mobility among scientists. This belief is nurtured by de Duve's long association with the Rockefeller Institute where he spends about half his time and will continue to do so. In fact, the Rockefeller clearly plays a godparent role to the ICP, acting as a general model, having been a temporary home for most of the senior staff at one time or another and providing two of the members of the Scientific Advisory Board.

Also in line with de Duve's encouragement of mobility will be his policy of only giving tenured posts to the most senior of newly appointed staff. Others will be taken on limited appointments of a few years, some on a fellowship programme that is about to start.

It remains to be seen how successful this policy will be at a time when even the best young scientists are not prepared to take untenured posts except as a last resort.

Professor de Duve is a modest man, courageous enough to try and build up an international institute in a city which, apart from becoming the Washington of the European Economic Community, has few outstanding attractions. His Nobel Prize last year has not only brought him fame and fortune but has been an exceptionally timely boost for the ICP. □

THOMAS JUKES



Evolutionists brought to book

THIS year is the fiftieth anniversary of the "Scopes Trial" in Dayton, Tennessee, when the issue of teaching evolution was argued between William Jennings Bryan and Clarence Darrow. The trial was showbiz, not, as some have thought, a public martyrdom of Scopes. It was put "on the air" in one of the first coast-to-coast network radio broadcasts. Scopes was fined \$100, this was paid by the *Baltimore Sun*, and Scopes left Tennessee to accept a graduate fellowship (a rarity in those days!) offered because of his new fame. The evolutionists got their story to the public. At the time, it seemed that Bryan succeeded in convicting the defendant but that Darrow triumphed ideologically. Half a century later, the story of the trial, as told by H. L. Mencken, sounds archaic to the young, and nostalgic to their parents.

But there is an uneasy feeling that, as far as school textbooks in the USA are concerned, evolutionists may have won the battle of Dayton, but not the war. For publishers live by selling books, and it is poor for business when parents object to a science textbook: the competition would gladly omit "controversial material" to win the contract. As an "evaluator" for the California State Textbook Commission, I was prepared to resist the opponents of evolution on the State Board of Education. I found, however, that there was very little mention of evolution in the books. Publishers will not prepare a special "California edition", so the result is that the national versions have little to say about the subject, with the notable exception which Professor G. G. Simpson has noted, of those published by Harcourt Brace Jovanovich. □

The "textbooks" published by the Creation Science Research Center of San Diego have plenty to say, however. Much of their content is artfully directed towards attempts to discredit geological findings and isotopic dating. Few physicists, palaeontologists and geologists wish to get into arguments emanating from those who truly believe in a special creation that took place instantaneously 6,000 years ago. But I think we may learn a simplified approach from the following famous exchange in the Scopes trial:

DARROW: Mr Bryan, do you believe that the first woman was Eve?

BRYAN: Yes.

DARROW: Do you believe that she was literally made out of Adam's rib?

BRYAN: I do.

DARROW: Did you ever discover where Cain got his wife?

BRYAN: No sir; I leave the agnostics to hunt for her.

Fifty years later, creationists are passing the hat for the latest of their expeditions to look for the remains of Noah's Ark on Mount Ararat. Surely if they wish to argue about geology, we are entitled to ask a few questions about the Ark, its dimensions, and the time schedule of the Flood. These are set forth with great precision in the Book of Genesis, in an account which also contains one of the most cherished of allegories—that of the dove of peace.

Our creationist friends, literal and unimaginative, are more concerned with looking for pieces of wood. Very well. Let us ask them a few questions about ecology in its most compact form—the living quarters on the Ark for all terrestrial species (including koala bears, *Plasmodia*, tapeworms and tigers), plus their food supply and maintenance for 400 days, in a volume of 44 000 m³. There is also the minor question of what happened to about 1.6 × 10⁹ km³ of water when the flood receded. This much water would be needed to cover the Earth to a depth of 3,000 m, an increase in water level that would be actually only about half that required to submerge and drown "every living substance . . . which was upon the face of the ground."

But I digress. In some religious sects, there is still a zealous opposition to teaching evolution, and the effects of this have been recently visible in the states of California, Texas and Washington. The subject of evolution is one of our great cultural heritages, which has now expanded to encompass biology in a single network of molecules, and the recent problems in the USA are a lesson to all of us to keep up-to-date on knowledge of the fossil record and the methods of isotopic dating of rocks. □

ASTRONOMY, cradled in superstition, and fed in infancy by the fears of kings, only became a science when it dissociated itself from astrology and restricted itself to matters capable of mechanistic interpretation. Other sciences, including most fields of medicine, have likewise weaned themselves from their infant diets of credulity and crude utility. In consequence, chemistry, mathematics and physics have become reputable disciplines whose general scope and aim could not be misrepresented in public documents without exciting wide comment.

Why is the genetics of man not accorded the standards of public scrutiny and stock-taking normally afforded to radioastronomy, high-energy physics, and the genetics of flies? It can hardly be mere difficulty, for the basic mechanisms of genetics are, in principle, far simpler than those invoked for stars and atoms, and the genetics of man is only marginally more remarkable than that of the fly. Until we can diagnose the cause of this contagious mediocrity we cannot expect any cure, and without some attempt at cure we cannot expect that workers of the calibre expected in those who listen to the skies, or look for portents in bubble chambers, will be attracted to this field. The reputed mediocrity of physicians, a belief widespread among those who are not physicians, is hardly a reason, for immunology, virology, and numerous other specialised fields in and around medicine are progressing well.

The reason must be in ourselves. While we can accept, after many generations, the peripheral isolation of the Earth, and even the Sun, the full implications of evolution and of natural variability are too much. Genetic disorders are rare, and only a minority are amenable to elimination, to prevention, or to treatment. They have been with us throughout our evolution, and most are likely to stay with us for as long. They require the same basic facilities for research, for diagnosis, for treatment, and even for elimination, as do other forms of disease.

Genetic disorders are, by definition, those to which the flesh is heir, and cover all disease. In practice it is convenient to use the word 'genetic' to cover those disorders consequent upon some hereditary unit, or pair of units, which is usually either sufficient, or necessary, for the manifestation of disease, as in the genic and chromosomal disorders. Victims include those who, by chance, were conceived without the alleles necessary for synthesising pigment in the eye and skin, or, by mischance, were conceived with the handicap of an extra chromosome.

Even here words can be confusing,

for albinos rarely have inherited any noticeable disorder from their ancestors, and those burdened by an extra chromosome are usually born to parents with a regular allocation. But the term genetic, implying an inborn qualitative or quantitative abnormality in their nuclear apparatus which is sufficient to cause distress, is useful and rarely ambiguous. Obviously, the environment will influence affliction, but, within any regular environment, any inability to cope with minor injury, sunlight, or conventional food can be a sufficient handicap to qualify for the term 'disease'.

Genetic facts?

Once the word genetic is extended to cover any inborn predisposition, whether to illness, to aptitude, or to crime, difficulties arise which can only be removed by a less casual use of words. A recent publication from the US National Institute of General Medical Sciences, National Institutes of Health, (*What are the facts about genetic disease?*, with the strange subtitle, *Most ubiquitous of all human maladies*) does nothing to lighten the genetic load of confusion, and its authors have done a serious disservice to those—many of whom are featured in photographs in this booklet—who are striving for an orderly application of genetic knowledge to man.

The book tell us (p. 6) that 15 million Americans have birth defects, and that 80% of these "carry true genetic diseases due wholly or partly to defective genes or chromosomes". The genetic load is then elaborated to cover 100,000 abortions and 40% of infant deaths; each of us (presumably US citizens) carries "between five and eight recessive genes for serious genetic effects" and each married couple has a 3% risk of having a genetically defective child. The opposite page is a little less exact with recessives, giving the range as "between one and ten", and shows some normal looking individuals and a superimposed pedigree of the extremely rare and variable Hartnup disease, stating that the normal siblings are at risk of transmitting it to their offspring—a risk too low to justify anxiety, or even the use of the word risk.

Next, disorders of infancy are shown to cause more lost years of life than disorders of the aged. Single gene defects are said to occur in 1.8 to 2% of all births. But the "overall incidence of recognised genetic diseases is 4.8 to 5.0% of all live births", a curiously exact estimate, but one which seems out by a factor of 10. We are told that

"authorities now estimate that seven to eight per cent of the US population is adversely affected by one or another form of heritable disease" (p 14) while the next page includes gout and diabetes as genetic diseases, which certainly helps the percentages. We are probably all potential diabetics. Heart disease emerges again on page 21: this time 20% of cases are attributable to one of three genes and 5% are polygenic (what about the other 75%; can anything be non-genetic and non-polygenic?). Those three unspecified genes are said to be the most common disease-producing genes. Estimates of hard cash are made with equal abandon. Death from Tay-Sachs disease is costed at \$35,000, which seems a lot unless it includes the artificial piping of both food and air to the dying. Earlier, the booklet states that "specific genetic factors are involved in approximately one-fifth of all heart attacks". What does this mean? Why not incriminate the Y chromosome, which is surely genetic, and is found in at least four fifths of victims of heart attack?

How can the economic custodians of medical research in a country which has such numerous claims to excellence in both aim and achievement, be associated with the publication of such a confused and contradictory document? For whom can it be meant? Why are the achievements made by other routes, such as safe blood transfusion, the 'cure' of rhesus disease by exchange transfusion, and its prevention by immunisation, not worthy of mention? Are not the triumphs of haemophilic therapy worth a sentence? The US decision to funnel funds into 10 centres seems strange, since excellence cannot be constrained, even in smaller countries; is this booklet for congressmen who think their constituents neglected? Or is it what the *Observer* calls "research by public hysteria"?

Genetic research needs money, and the excellence of this research in the US would seem to have suffered even more than that in most other countries during recent years. A pre-occupation with costing, with short term aims, and the throwing around of wild estimates of such measures of the genetic load as the number of recessives per gamete, or the number of dollars per death, does little to reassure admirers of American genetics that the future will be worthy of the past.

It is tempting to dismiss this as a mere manifestation of bureaucratic immaturity, hardly worthy of comment. The matter is however, too important to be glossed over in this way. There is nothing more important than the orderly survival and civilisation of our own species.

J. H. Edwards

international news

THE absence of an official statement from the World Meteorological Organisation (WMO) after the recent meeting of its working group on stratospheric and mesospheric problems is being interpreted as an indication that WMO sees no danger of imminent disaster caused by the depletion of the Earth's protective ozone layer. This is in spite of calculations presented to the group to the effect that long term depletion of stratospheric ozone by the continued release of freons at 1972 levels could amount to more than 10%, and that ultraviolet radiation reaching the ground could increase by 2% for every 1% decrease in ozone. The group apparently wrote into its deliberations that it was working on a "factor of uncertainty" of about 2, which, at worst, would indicate a 20% depletion of stratospheric ozone, with a corresponding increase of 40% in radiation levels. This would be serious by any estimate.

The working group on stratospheric and mesospheric problems was set up under WMO's Commission for Atmospheric Sciences, and included many of the world's leading experts in this field: physicists, stratosphere chemists, experts on ozone, on the physical and chemical behaviour of the stratosphere,

WMO silent on ozone depletion

from Peter Collins, Geneva

and those concerned with evolving models for the study of what goes on in the stratosphere. The group was concentrating principally on the chlorofluoromethanes (freons) resulting from the use of certain aerosols and certain types of refrigerator, and which have recently been the subject of alarm, especially in the US. It is the first time that these substances have been considered by WMO at this level and it is indicative of the increasing attention being paid there to the broader environmental aspects of air pollution.

As summed up by a participant, the meeting concluded that calculations on an average, world-wide basis, ignoring latitude and the longitude and using one-dimensional models, indicate that the present anthropogenic contribution to the depletion of ozone by freon 11 (CFCl_3) and freon 12 (CF_2Cl_2) already in the stratosphere is 0.5 to 1.00% of the total amount of ozone. The long-term steady state effects of continued release of these substances at 1972

world rates would result in about a 10% depletion with an average factor of uncertainty of about 2. The effects of this 10% on the thermal regime of the stratosphere might be noticeable but are still not clear; the consequences of any such changes on tropospheric weather systems are likewise uncertain. It has also been estimated that a 1% decrease in the total ozone amount would permit a 2% increase in ultraviolet radiation at ground level, always assuming clear weather.

The group also discussed other pollutants such as bromine and nitric oxides, but it has been pointed out that the freons arising from human activities at the Earth's surface are very much more serious than anything that could happen from the use of aircraft, including nitric oxide emissions which are of such concern to the anti Concorde lobby. No official report of the meeting is likely to be forthcoming for some months, and even then it is likely to express the conclusions with great caution. One reason for this is that we still know very little about what goes on, chemically and physically, in the stratosphere. Here, the group did express itself clearly in agreeing on the need for more data from observation. □

NOT quite two years after the publication of its third and final report recommending major changes in the formulation and implementation of science policy in Canada, the Senate's special committee on science policy is preparing for an investigation of how effectively those changes have been carried out.

Senator Maurice Lamontagne, the committee's chairman, says letters went out in September to the Ministry of State for Science and Technology (which was created in part as a result of the committee's recommendation) and the Science Council of Canada, asking them to prepare briefs for a new set of hearings to begin in November.

Since the publication of the last volume of its report in the autumn of 1973, the committee has been concerned with planning a major conference on future research for decision makers at various levels of national life to attend. That involved preparing background papers for an area of interest largely neglected by other institutions in Canada. The committee also proposed establishment of two

institutions: a Canadian Centre for Future Studies and a commission called Futures Canada.

Much to its obvious chagrin, the federal government decided last summer to accept the thrust of the Senate committee's recommendations, but to

Lamontagne revisited

from David Spurgeon, Ottawa

take the matter out of its hands and entrust it to the recently formed Institute for Research on Public Policy.

This left the committee without a job.

Senator Lamontagne's response was to propose a return to its former mandate, "not to start a new inquiry but to uncover the areas where our demand for change and improvement has been met with minimum compliance, or simply with autocratic and bureaucratic rejection."

Even before the new hearings begin,

the Senator is less than pleased about governmental response to his recommendations. He sees MOSST (the science ministry) as possessed of "suicidal" tendencies (some 30 senior employees were let go recently), and he finds no technological strategy developed by the Department of Industry. "Government support for science, technology and innovation," he told the Senate, "continues to be inadequate, uncoordinated and wasteful."

The Science Council of Canada may welcome the committee's new activity, because it seems to have been eclipsed more and more as a result of the creation of MOSST. But it is difficult to believe either the government or MOSST will be exactly overjoyed. The ministry seems to have been slipping slowly into oblivion in recent months, and just as the new part-time minister, C. M. Drury (who also held the portfolio of public works) had begun to counteract this drift with a public definition of its role, he found himself appointed interim finance minister as a result of the resignation of the former minister, John Turner.

Pauling comes in from the cold

PRESIDENT Ford last week invited 13 scientists to the White House to receive the National Medal of Science, the most prestigious scientific prize offered by the federal government. For one of the recipients, Linus Pauling, the event had added significance, for it marked the end of a long and bitter period during which Pauling had been virtually ostracised by the White House for his outspoken political opinions and his opposition to nuclear weapons.

Although he has received almost every possible scientific accolade, including two Nobel Prizes, Pauling has suffered a number of direct reprisals for his political views, and he has repeatedly been ignored when the National Medals of Science have been handed out. The latest such rebuffs came during the Nixon Administration when Nixon, who was not known for honouring his enemies, twice overruled Pauling's nomination for the prize.

Pauling's political troubles began in the early 1950s, during the anti-communist witch hunts which culminated in the rampagings of Senator Joe McCarthy. Accused of being a Communist sympathiser, he was denied a passport in 1952 to travel to Britain for a meeting of the Royal Society, and he was again refused a passport a year later. He was also refused a government research grant in the early 1950s for political reasons.

Such overt reprisals lessened a little after he was awarded the Nobel Prize for chemistry in 1954, in recognition of his pioneering work in unravelling the nature of the chemical bond. He was, at least, granted a passport. But Pauling continued to needle successive Administrations by attacking nuclear weapons testing, foreign policy and, in the 1960s, the Vietnam war. Although his actions brought rebuffs at home, Pauling's political activities brought him the Nobel Peace Prize in 1963 and the International Lenin Peace Prize in 1971.

There was, however, one occasion on which the White House temporarily suspended hostilities against Pauling. Soon after he was elected, President Kennedy invited Pauling, along with every other American Nobel Prize-winner, to a White House dinner. Pauling accepted, but before attending the event, he led a protest demonstration outside the White House gates against nuclear weapons testing.

The citation which accompanied his National Medal of Science states that the award is "For the extraordinary scope and power of his imagination, which has led to basic contributions in such diverse fields as structural chem-

Pauling at the White House with a friend (pic: Washington Post).

istry and the nature of chemical bonding, molecular biology, immunology, and the nature of genetic diseases". Pauling, who is now 74, accepted the prize with a broad grin, accompanied by the clicking of a multitude of cameras.

Other recipients of the award were Britton Chance (University of Pennsylvania), Erwin Chargaff (Columbia University), James Neel (University of Michigan), James Shannon (former Director of the National Institutes of Health), Rudolf Kompfer (Stanford University), Ralph Peck (University of Illinois), Abel Wolman (Johns Hopkins University, now retired), Kurt Godel (Institute for Advanced Study), Nicolaas Bloembergen (Harvard University), Paul Flory (Stanford University), William Fowler (California Institute of Technology) and Kenneth Pitzer (University of California, Berkeley).

● At the annual International Astronautics Federation congress this week in Lisbon, the Guggenheim International Astronautics Prize, awarded by the International Astronautics Academy, goes to Oleg G. Gazenko, now Director of the Institute of Biomedical Problems in Moscow. More than anyone else in either the US or Soviet space programmes, he has made a concerted attack on the daunting problems of placing and maintaining man in space—and it is now hard to remember that before 1957 many

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physiologists were convinced that such a venture would only prove a costly catastrophe. The line of development conceived and carried through by Gazenko and his colleagues can be considered to have culminated in the effective compromise of the Apollo-Soyuz joint test project, in which the Soviet life support philosophy and that of NASA were blended rather than unilaterally bent.

Oleg Gazenko's involvement with the physiological problems of space flight and life support for long manned space-flights goes back beyond 1957, to the time when the first Soviet physiological experiment was carried out in space—involving the dog Laika in the second *Sputnik*. The choice of the dog as the test animal for pioneering *in vivo* experiments in space may be attributed to Gazenko, although it conformed equally with classical Pavlovian behaviourism favoured by the Soviet medical establishment.

Since the mid-1960s Gazenko has been in the thick of the project (finally agreed in principle in 1964) for joint publication of US and Soviet space medical and biological experience (*Foundations of Space Biology and Medicine*). Recently, American research and competence in space biology and medicine has more than caught up and it has assumed for a year or two that Gazenko had lost status. This award should set the record straight. □

AN extraordinary period of expansion in the life of Israeli universities is coming to an end. After a quarter of a century during which student enrolment has increased almost 40 fold (in contrast to a five-fold increase in the general population), it is now up by only 5 or 6% a year. And severe financial problems have virtually brought an end to the spate of multi-million pound building projects so characteristic of the last 25 years.

Not all universities have been affected in the same way by the new situation. Though the 50 year old Hebrew University of Jerusalem is actually expecting a decline in enrolment this year, the newer institutions of higher learning in Haifa, Tel Aviv and Beersheba are still growing, albeit at a slower pace than previously.

Off the record, some professors argue that Israel does not need even its existing seven institutions of higher learning to handle the current student population of some 60,000 (which gives Israel about the same percentage of university students as the countries of western Europe). Since, however, it would be politically impossible to shut one down, all the Council for Higher Education could do was to ban the establishment of new universities (in the foreseeable future) and to crack down on plans to create new faculties at existing universities, particularly where they duplicate programmes already available elsewhere in Israel.

Reaction to the situation has varied from campus to campus. The President-elect of the Weizmann Institute, Professor Michael Sela thinks that Israeli institutions of higher learning should, in the circumstances, "use their shrinking resources to raise academic standards rather than add floor space". And Professor M. Z. Kaddari, until recently Rector of Bar-Ilan University, wants to cut down on the intake of students, not only because of budgetary problems but also "to avoid the creation of a disillusioned academic proletariat".

Naturally enough, the President of the Technion Institute of Technology, Amos Horev, believes that, regardless of the number of students, a greater percentage of them should be studying engineering lest a shortage of engineers frustrate Government plans to double Israeli industrial output by 1980.

Whatever the logic of Horev's argument, priorities in the sphere of higher education will have to be more clearly defined than they have been in the past, when money seemed to be available for everything at once. The boom is over.

● Despite their financial problems, or possibly because of them, institutions of higher learning are strengthening their ties with local industry. A recent con-

sequence of such cooperation is the Microviscosimeter MV-1, an Israeli instrument expected to facilitate the early detection of leukaemia, which was this week named one of the 100 most innovative products of the year by *Industrial Research*, a US magazine.

The instrument was developed by the Elscint Company of Haifa on the basis of studies carried out by young researchers Michael Inbar and Meir Shnitzky of the Weizmann Institute's Laboratory of Membranes and Bioregulation. It grew out of an

Letter from Israel

from Nechemia Meyers

apparent correlation discovered by Dr Inbar and Dr Shnitzky between the level of cholesterol in the cell surface membrane and the blood, and the development of leukaemia.

Named Israel's Outstanding Exporter of 1974 for its large overseas sales of medical and laboratory equipment, Elscint is particularly proud of the fact that its MV-L is sharing honours this week with instruments created by a group of international giants.

● Another achievement of local, science-based industry unveiled recently is a mobile pilot plant for processing sewage that provides as end products both purified water (suitable for irrigation and almost suitable for human consumption) and concentrated algae for use as high-protein animal feed.

Built by the Membrane Division of Israel Desalination Engineering Ltd (IDE), under the supervision of Dr S. Sachs, the installation, unlike treatment plants now in operation, does not require the introduction of algae-killing lime or alum into its oxidation pools. Instead, it concentrates and purifies the sewage by low pressure ultrafiltration, using membranes developed specifically for the task.

It is not yet possible to estimate the cost of building and operating IDE units on a commercial basis, but an important factor in costs—the energy input required—is relatively low because the purification process can be carried out at a pressure of 7 or 8 atmospheres, as compared to 60 or 70 atmospheres in conventional reverse-osmosis systems. Moreover, the low pressure permits the unit to be built with less expensive materials than would otherwise be required.

When expenses are calculated one must also consider the fact that the algae 'left over' should bring in substantial sums of money.

Economic considerations in Israel

may be rather different from those of other countries, as additional water supplies are urgently required here, almost regardless of cost. But both the Treasury and the taxpayers would be most grateful if recycling a larger part of Israel's sewage made it possible to delay the construction of large scale and very expensive desalination plants. ● A more controversial scheme for increasing water supplies has come from the Ministry of Agriculture's Soil Conservation and Drainage Department. Ministry experts claim that transforming 85,000 acres of shrub-covered Galilee hillsides into pasture land would save 85 million cubic metres of water because grass requires less moisture than shrubs and the "surplus rain" would seep down into the aquifer. Moreover, they add, the new grasslands would provide enough forage for raising 24,500 cattle each year, as well as creating an excellent recreation area.

The scheme has, however, been severely criticised by, among others, Professor Zeev Raveh of the Technion Institute Faculty of Agricultural Engineering. "If the plan were implemented," he declares, "it would supply the country with very little additional water or meat, and, far from creating a new recreational area, it would gravely endanger the ecological balance of one of Israel's few remaining focuses of natural beauty and rich biological diversity."

Pointing out that the Ministry of Agriculture plan has so far only been tested on a carefully tended, one and a quarter acre plot, Professor Naveh doubts very much whether the lessons learned there are applicable over a much wider and more diverse area. He notes, for example, that uprooting shrubs is a very expensive process if it is to be effective (costing several times more than was estimated by Ministry planners) and, he adds, there is no certainty that the new vegetation that takes over will be suitable for grazing purposes.

Though a few months ago it was planned to implement the Ministry of Agriculture scheme at the rate of 8,500 acres a year, public protests have prompted the Government to undertake further studies before uprooting any shrubs on a large scale. At the same time, measures will undoubtedly be taken to foster the multipurpose use of Israel's extremely limited areas of land. Professor Naveh, for his part, thinks that they must be carefully designed to suit the requirements and possibilities of each site so as to achieve a maximum degree of economic benefit (in the form of increased water yields, grazing land and timber) while ensuring that key elements in the ecosystem are not undermined.

correspondence

Moon

SIR,—One must assume that at the beginning of each year the Editor of *Nature* writes in his diary, in the appropriate place, "Be sure to include an article designed to discourage readers from attending the International Conference on the Unity of the Sciences". Having attended last year's ICUS conference, I can vouch for the fact that the majority of the eminent names mentioned in the advance publicity did in fact attend the conference and make contributions. As far as I am aware, no-one present was dissatisfied at the proceedings, and at the end I was left with the impression that the conference had succeeded in achieving the aim of any conference, namely of allowing free exchange of ideas on important topics.

Regarding the sponsorship of the conference, from his opening address I gained the impression that at any rate some of the Rev. Moon's motives are at least as worthy as those of such well known conference sponsors as the armed forces. As to the rest, news (as reported by you) is capable of being distorted in many ways, but whatever the true situation may be, I find it difficult to accept from a reputable scientific journal something which one cannot interpret as anything other than a deliberate attempt, for ideological reasons, to sabotage a conference by discouraging potential participants from attending.

Yours faithfully,

B. D. JOSEPHSON

Cambridge, UK

Silly in the head

SIR,—Regarding the "silly season for newspapers" as discussed in *Nature* (August 28), how about the headline for a recent Independent Television News at Ten—"British scientists discover origin of Universe"?

Yours faithfully,

JOHN WOODSIDE

Cambridge, UK

SIR,—Herewith some suggestions for your silly headlines list:

Britain fastest in entropy acceleration studies—official
Plutonium for Arabs in genetic manipulation research deal
Pugwash scientists named in SALT talks sell-out allegations
Ice-age predictions snowball as funds frozen

Nature readers in shock titles scandal
—alleges Longford

Yours faithfully,

BERNARD MEARES

Copenhagen, Denmark

Stop metrication

SIR,—On August 28 you referred to the pointless advance of metrication. There is one area where good sense has a foothold, even in France. Whether in Notre Dame or the humblest village church, organ pipes are still measured in feet. I could not think of foundation stops of 2.4 m or adding the brilliance of a 0.3 m rank.

Common sense still prevails over logic in one area—I am sure that there must be others.

D. C. HARDWICK

London, UK



CAB directors

SIR,—It is unfortunate that your correspondent in his article on the Commonwealth Agricultural Bureaux (August 14) spoiled an otherwise factual and informative piece by an inclusion of quite unmerited criticism of CAB directors.

As your correspondent says "the bureaux have maintained and justified their reputation for providing the world's best and most comprehensive source of information in the fields they cover". The reputation has been gained not by the central management but by the individual bureaux under the management of the directors. That changes are needed to modernise the bureaux structure is acknowledged by the staff, and the Institution of Professional Civil Servants (IPCS) has put forward on their behalf proposals which we hope the CAB management will adopt but which will be rather

different from those proposed by the Civil Service Department review team.

The CSD review team made recommendations which implied criticism of the central management rather than of the staff or the directors of bureaux. The misuse of computer printing, resulting in an increase in the time taken to publish abstracts as compared with the time when directors were completely responsible for publication, is an example. Clearly there is room for improvement in the management of computer services. Certainly to the extent that it is possible, collaboration with other agencies doing similar work is desirable. But your correspondent is wrong in assuming that there is not already collaboration with AGRIS; the bureaux already scan UK journals for AGRIS and make no charge for this service.

The report of the review team stress on the economies in staffing which would result from operating the abstracting and editorial services on a commercial basis. We believe that this could be achieved only by lowering the standards of the service and this would seriously reduce its value to its users. An important feature of the CAB service is its abstract enrichment and this could not be maintained if commercial viability were the first consideration. The review team made no attempt to test its assumptions by market survey. Enquiries we have made from a necessarily small sample of eminent scientists in the agricultural field have all met with the response that the CAB abstracts must be maintained at their present high quality if they are to be of value. By all means let us have additional effort to market the CAB's products (and the staff will not be greatly concerned whether this job is described as "marketing" or "circulation and services") but the article for sale must be first class.

Whatever changes are made in procedures and staff structures they will be successful only if they have the whole-hearted cooperation of the staff. A lowering of standards would lead to loss of job satisfaction for the staff and a drop in morale. Staff morale will not be improved by suggestions such as those made by your correspondent that the staff are responsible for the difficulties which are solely attributable to bad management.

EDWARD HEWLETT

IPCS, London, UK

news and views

LESS than a year after their discovery it was widely accepted that pulsars are rotating neutron stars, with high magnetic fields and consequently with a very energetic co-rotating magnetosphere. Progress beyond this basic interpretation has been slow, not through a lack of experimental data but through the sheer unfamiliarity of the physics involved in these strange objects. The solid state physics has to deal with densities approaching $10^{15} \text{ g cm}^{-3}$, with a composition varying from a neutron superfluid to a crystalline crust mainly composed of iron nuclei. The very strong magnetic field is sustained by the superconductivity of the neutron star, and itself modifies the crystalline structure of the crust. Conditions in the magnetosphere are even more bizarre, with complete charge separation and vacuum spark gaps as possible features.

The difficulty in interpreting the radio pulses, which provide our only observational material on nearly all of the pulsars, is that they originate in only a local region of the magnetosphere, and their radiation processes are limited indicators of the behaviour of the rest of the neutron star. Furthermore, they have very complex structures, which we cannot easily interpret without knowing more about the radiation process and the precise location of the emitter in the magnetosphere.

Recent work on pulsars at Jodrell Bank has used the timing of the radio pulses to investigate the slow-down of rotation of the neutron star. The

Physics of pulsars

from F. G. Smith

slow-down is a consequence of radiation from the rotating dipole magnetic field, so that this investigation was using the radio pulses as an indicator of conditions within the star. Surprisingly it led to the suggestion that the magnetic field decays with a time constant of a few million years, which is in conflict with present theories of the superconducting interior of neutron stars.

Another investigation, also related to the age and evolution of pulsars, but which refers to the magnetosphere rather than the star itself, is reported in this issue of *Nature*. Ritchings and Lyne (page 293) have found a new orderliness in the apparent disorder of the structure within radio pulses. Some pulsars, and particularly the older ones as shown by the previous investigation, have "drifting sub-pulses". An individual radio pulse in these pulsars consists of one or two narrow sub-pulses, which can be seen in several consecutive pulses but at slowly varying times within the pulses. The "drift" in time can be either earlier or later, although until recently it was thought that all drifts were earlier. The remarkable observation now reported is that these two cat-

egories are simply divided between those pulsars with slowly lengthening periods, which drift earlier, and those with rapidly lengthening periods, which drift later.

The most important conclusion must be that there is after all some simple classification and evolution in the pulsars, whose behaviour may perhaps be regulated mainly by the strength of their magnetic fields. We do not know at all how the drifting itself occurs, although there have been some interesting suggestions about the drifting of discharge paths across a spark gap near a magnetic pole. But we can at any rate rule out those theories which allow only one direction of drift, or those in which the direction was regulated by the aspect of the rotating star as seen by the observer.

The physics of the emission process is not revealed by the drifting phenomenon, since this may occur in a region remote from the emitter. For example, drifting in a spark gap at the poles provides a lateral movement in a stream of particles, which then sweep across an emitting region which might be close to the velocity of light circle. There is some evidence in favour of this location for the emitter from the work on pulsar slowdown rates, which indicate that the pulsars stop emitting when the magnetic field in that region falls below a critical value. On present evidence, the behaviour of pulsars seems to be completely dominated by the strength of the magnetic field.

SEAMOUNTS are active or extinct conical or flat-topped ocean floor volcanoes with elevations of at least 1,000 m, the majority not rising above sea level. They occur throughout the world's oceans but are particularly associated with the Pacific where, according to Menard (*Experientia*, **15**, 205; 1959), there are some 10,000. Several hundred of the Pacific volcanoes form linear chains (the best-known of which is the Emperor-Hawaiian chain) which are widely believed to result from the motion of the Pacific plate above hot spots with roots in the mantle. Most seamounts, however, are apparently individual bodies located at random on the ocean floor, although they tend to lie in clusters or bands.

As long as the oceans were thought

Do seamounts form near ridges?

from Peter J. Smith

to be permanent, the points of origin of seamounts posed no particular problem; volcanoes erupted on the ocean floor and thereafter remained fixed in position. But moving sea floors have introduced an ambiguity. Leaving aside the small proportion of seamounts aligned in chains, did the rest originate at random locations by some intraplate volcanic process or did they erupt near oceanic ridges and spread away to cover the ocean floor? As the number of seamounts per unit area of ocean floor does not seem to increase

away from spreading ridges (which means that the density of seamounts is no greater in older than in younger sea floor), the random hypothesis seems the less likely; but this can hardly be said to be definitive evidence.

An obvious way of settling the matter would be to date both seamounts and the surrounding ocean floor; a seamount embedded in ocean floor of comparable age would then imply ridge formation whereas discrepant ages would imply random volcanism. Unfortunately, experiments along these lines have provided conflicting evidence. Fisher *et al.* (*Science*, **160**, 1106; 1968), for example, found that three seamounts near the East Pacific rise were apparently up to 40 Myr younger than the underlying crust. The age of the

crust was poorly defined at that time, however, and a more recent tectonic reconstruction of the region by Herron (*Geol. Soc. Amer. Bull.*, **83**, 1671; 1972) has reduced the seamount-ocean floor age difference to between 5 and 6 million years. An even closer age agreement was obtained by Ozima *et al.* (*Earth planet. Sci. Lett.*, **8**, 237; 1970) for three Pacific seamount dredge samples (two Cretaceous, one Pliocene). Dymond and Windom (*Earth planet. Sci. Lett.*, **4**, 47; 1968), on the other hand, found that although two seamounts resting on Cretaceous crust southwest of Hawaii were also Cretaceous, a third was less than 1 Myr old.

In an attempt to resolve such conflict, Clague and Dalrymple (*Geophys. Res. Lett.*, **2**, 305; 1975) have now dated four seamounts in the central North Pacific—two from the Hawaiian ridge (Wentworth Seamount and Necker Island) and two from the Musicians Seamounts (Rachmaninoff and Khatchaturian). The samples from Necker and Wentworth are, respectively, at least 60 and 40 Myr older than the adjacent Hawaiian bodies as predicted according to the hot spot-plate motion hypothesis, and thus conflict with the concept of a Hawaiian chain ageing progressively westward. Rather than taking these results to disprove the age progression, however, Clague and Dalrymple propose that Wentworth and the northern part of Necker are Cretaceous seamounts that predated the formation of the Hawaiian chain and later became incorporated within it. Indeed, on the basis of seamount densities within the Pacific in general and in the area surrounding (but excluding) the Hawaiian chain in particular, the incorporation of at least this number of older seamounts is to be expected.

Assuming that Wentworth and Necker are unrelated in origin to the main Hawaiian chain, therefore, it only remains to compare their ages with those of their respective areas of surrounding ocean floor. Unfortunately, this is not easily done, largely because of the lack of magnetic anomalies in the vicinity of the Hawaiian ridge. From available fossil and radiometric age data and by extrapolating magnetic data from further afield, Clague and Dalrymple nevertheless conclude that Necker Island and possibly Wentworth Seamount are only slightly younger than their underlying crusts; likewise, the Rachmaninoff and Khatchaturian Seamounts, and two other Pacific seamounts previously well dated by other workers. Thus although the data are few and one or two discrepant seamount-crust ages still remain (notably that of Dymond and Windom), Clague and Dalrymple propose that most Pacific seamounts did indeed form on or near the East Pacific rise. □

Cyclotrons of all shapes and sizes

from E. G. Michaelis

The Seventh International Cyclotron Conference was held in Zurich on August 18–22 at the Swiss Institute for Nuclear Research.

THOUGH advancing into middle age and occasionally overshadowed by its Gargantuan progeny the cyclotron continues to develop vigorously, and after a series of facelifts its original passport photograph still current in physics textbooks is anything but a true likeness. The art of cyclotron construction remains challenging and cyclotrons serve a wide spectrum of scientific disciplines. Its devotees are still growing in number and budgetary restrictions have not diminished their enthusiasm or their ingenuity.

The Swiss Institute for Nuclear Research (SIN) welcomed more than two hundred participants from twenty-one countries with Swiss hospitality, and the report of the successful commissioning of the world's most powerful isochronous cyclotron by the Institute was a major contribution to the conference. News of similar successes achieved with large machines in Vancouver and Indiana proved that present designers can meet almost any challenge in this field.

Most of the 90-odd cyclotrons forming the subject of the conference are isochronous and sector focusing. Like the classical cyclotron they operate at constant orbital frequency but maintain this to relativistic ion energies by a magnetic guidefield increasing with orbit radius. Edge focusing between sectors of higher and lower field provides the axial stability which is absent

in a cylindrically symmetrical, radially increasing field.

In Spiral Ridge machines the sectors are curved to increase axial focusing, and in Split Pole cyclotrons the magnet poles are restricted to the high-field regions to increase the azimuthal variation of the field. The largest machines of this kind, the 590 MeV proton ring at SIN and TRIUMF, and the 520 MeV H^- accelerator at Vancouver combine both features. The magnetic fields in sector-focusing cyclotrons are subject to close tolerances and the ions are accelerated rapidly by high electric fields in two or more accelerating gaps to minimise the effects of local instabilities. Since technology and computer-aided design have made it possible to meet these conditions cyclotrons have become ever more powerful and versatile, many permitting the acceleration of a wide range of ions to prescribed peak energies. While problems of orbit stability are therefore largely resolved, those of ion sources, injection and extraction still limit performance and received much attention at the conference.

The growing interest in heavy, multi-charged ions calls for new ion sources to avoid the present need for several accelerators in cascade with ion stripping to higher charge states between successive stages. Reviewing the field J. Arianer (IPN, Orsay) showed that electron beam sources furnish more intense beams of highly stripped ions than the conventional PIG source or present laser sources.

Large and complex sources cannot be mounted in a cyclotron, and polarised, H^- and heavy ions are usually injected after some pre-acceleration. Only large split-pole machines permit mid-plane injection while particles are axially injected and deflected into the mid-plane of smaller machines. Interesting progress in mid-plane trochoidal injection however was reported by W. van Kampen (Delft Technical University). 200 keV protons are made to precess along a sector edge towards the middle of the cyclotron, where a suitably perturbed field gradually centres the orbits.

Ion extraction becomes more difficult as the separation between successive turns decreases with increasing particle energy, but notable successes in the extraction of energetic ions were announced at the conference. H. Willax (SIN, Switzerland) reported that more than 90% of the 590 MeV protons in the SIN accelerator can be extracted thanks to an energy gain exceeding 2 MeV per turn. J. R. Richardson (TRIUMF, Vancouver) described the simultaneous extraction of two proton beams of different energies from the large H^- accelerator and CERN achieved an extraction efficiency ex-



A hundred years ago

The celebration of the fiftieth anniversary of the opening of the first railway between Stockport and Darlington is attracting the notice of the French papers. Baron Charles Dupin, who published his celebrated work on Great Britain in 1826, described to the Institute that locomotives could never move, owing to the weakness of their hold on the rails, and that the use of horses could not be dispensed with. Baron Charles Dupin's reputation was so great that the truth of the statement was taken for granted. from *Nature*, **12**, 483; September 30, 1875.

THE trend towards simplifying ecosystem complexity for comparative purposes by the use of common energetic terms often causes confusion by obscuring basic principles. The 'standing crop' of an ecosystem is one such term which indicates how much energy there is in the system at any one time. But the total amount of, say, primary production in energy terms available may not be the same as the amount actually available to the herbivores which feed upon it. Thus the figure for standing crop may be a red herring to anyone wishing to determine whether food is limiting in the ecosystem. A rule of thumb guide to consumption of grassland vegetation by herbivores is that rarely is more than 10% of annual primary production actually eaten (Wiegert and Evans, in *Secondary productivity of terrestrial ecosystems*, 449 (edit. by K. Petruzewicz) (Polish Academy of Sciences, Warsaw, 1967)), and in many instances 2% is nearer the mark (Grodzinski, *Acta theriol.*, **16**, 231; 1971). On the face of it, therefore, food would not seem to be a limiting factor to herbivore populations.

The fallacy in this argument is to equate primary production standing crop with food. Food is more than just energy, and for most large ungulates a constituent of major importance is crude protein. Bredon *et al.*, among others, concluded that when the crude protein content of forage dropped to less than 4% dry weight, plant material ceased to function as food and ungulates feeding on it started to lose weight (*J. agric. Sci. Camb.*, **61**, 101; 1963). Death does

not follow immediately and cattle kept on low quality grass continue to feed, but they face mounting physiological stress.

A careful demonstration of the hazards inherent in the standing crop approach to ecosystem dynamics is provided by Sinclair's recently re-

Standing crop $\neq$ food

from our Animal Ecology Correspondent

ported studies on limiting factors in the Serengeti (*J. Anim. Ecol.*, **44**, 497; 1975). He points out that herbivores—ungulates, small mammals and grasshoppers—consume 27%, 38% and 14% of the annual primary production of tall grassland, short grassland and kopje country respectively. This leaves a large portion of the annual production for detritivores and for fuelling fires. Why is it not used to support higher populations of herbivores? The answer is that for a part of the year it is unavailable as food, much of the protein and carbohydrate in the leaves having returned to the roots. Sinclair's figures show that from a spring level of 8% crude protein, dry season grass contains between only 1% and 3%. In the tall grassland, food required by herbivores is in excess of that available for the duration of the dry period—July to September. This is associated with a drop in small mammal and grasshopper populations and hence foraging pressure, but is compensated for by an increased intake by ungulates. Monitoring bone

marrow fat levels in wildebeest over the year reveals that they fall slightly during the dry season indicating some degree of physiological stress experienced at this time. In the kopje country where ungulates were seldom seen, herbivore food requirements outstripped production for a much shorter period largely because of the rapid decline in rodent and insect numbers. This strategy for accommodation to sudden unavailability of food is open only to small herbivores with fast growth and reproductive rates and is barred to ungulates who must tighten their belts and just make do.

Hairston *et al.* and other workers have suggested that since herbivore populations consume so little of the primary production they cannot be limited by lack of food, and so must be limited by parasites, predators or disease (*Am. Nat.*, **94**, 421; 1960). The available evidence simply does not support this hypothesis. Sinclair is right to point out that an important implication of Hairston's hypothesis is that interspecific competition and niche diversification is precluded. There is no evidence that this occurs and plenty that it does not. Furthermore, consideration of mean annual values hides fluctuations not only in crude protein content but perhaps also in trace elements and other specific nutrients at certain times of the year. Availability of primary production as food is also affected by the physical correlates of feeding; crushing, despoiling and dropping. It is also affected by the nutritional and other needs of different age and sex classes in the populations.

ceeding 70% in its reconstructed synchro-cyclotron.

Work on heavy-ion acceleration was reported from several laboratories. A 25 MeV Tandem Van-de-Graaff under construction at Oak Ridge National Laboratory will inject ions into an existing cyclotron and will raise its energy to 800 MeV for ions of mass 160. M. Gouttefangeas (CEA, Saclay) described a recently authorised French project in which three cyclotrons in cascade will accelerate the heaviest nuclei to 10 MeV per nucleon.

The large, split-pole cyclotrons favoured at present make for easy acceleration and extraction but require much steel and expensive buildings. Much more compact constructions can be achieved by using superconducting coils to provide fields of order 5T and the conference learnt of projects for such "superconducting" cyclotrons at the Lawrence Berkeley Laboratory and at Chalk River, Canada. Michigan State University is constructing a super-

conducting cyclotron magnet in order to study the problem posed by this new technique.

A tantalising project for a 100 mA 800 MeV proton cyclotron was described by Yu. N. Denisov (JINR, Dubna, USSR), but its estimated radio-frequency power of 76 MW made it seem somewhat unrealistic.

Although primarily a gathering of accelerator physicists and engineers the conference devoted several sessions to the applications of cyclotrons and the range of topics proved the cyclotron to be a maid-of-all-work. The measurement by surface activation of abrasion in slurry-carrying ducts or of railway wheels, the determination of the fluorine content of a cup of tea by charged particle activation analysis, the study of nuclear level structures and excitation functions, radiation damage in reactor materials, the production of isotopes for medicine, neutron therapy, proton radiography and surgery are only a few of the topics covered by

various contributions.

M. M. Kligerman (University of New Mexico) reported on a comparative test of a treatment of skin melanoma by pions and X rays. The dramatic superiority of pions is a challenge to accelerator designers. A panel discussion on "Accelerators for Hospitals" reflected the difficulties, but tended to favour a proton-accelerator of variable energy.

The conference participants were able to visit the cyclotrons and experimental installations of SIN where a biomedical pion-channel and an eight-metre superconducting solenoid providing record fluxes of muons were of particular interest.

Professor M. S. Livingston, the co-inventor of the cyclotron, reviewed its early history in a delightful and informative after-dinner speech. Dr R. Wideroe, the inventor of resonant acceleration also attended the conference and one may hope that the two veterans were pleased with the development of their brain-child. □

Satellites to the fore

from W. H. McCrea

EARTH'S satellite the Moon is not only the astronomical body other than the Earth about which most is known; it is also the astronomical body about which knowledge has increased most rapidly in recent years—thanks to the spectacular successes of the Apollo and Luna missions. Even more recently, Pioneer 10 and 11 have provided invaluable new information about the Galilean satellites of Jupiter. At the present time, therefore, astronomers are more satellite-minded than ever before. Above all, they are interested in the origin of satellites because this may provide a check upon, or even a clue to, the origin of the planets. After all, astronomers have only the one planetary system to study, whereas they have six satellite systems; so it is reasonable to think that the satellite problem might be solved before the planet problem.

T. Gold has reviewed the situation and made a number of suggestions (*Icarus*, **25**, 489–491: 1975). He begins by asserting that all planets for which satellite orbits would be stable do possess satellites, claiming that for Mercury the solar perturbation is too large, that the retrograde spin of Venus would cause satellites to spiral into the planet through tidal friction (but I think he means that the slowness of the spin of Venus would render it retrograde as seen by any possible satellite), and not mentioning Pluto. Anyhow, Gold is obviously correct in remarking that satellites are a common feature of the Solar System, and this stimulates him to seek a process of formation of the widest possible applicability. In general terms, he appeals to the dynamics of grains in the presence of massive gravitating bodies (Sun and planets) and of a resisting medium (neutral and charged particles and photons). If initially a lot of grains are moving at random in a gravitational field under the action of no other forces, the motion remains random, that is, it is conservative, and there is no tendency for the motion or space-distribution to become systematised in any way. But matters are different if dissipative forces play a part. If there are inelastic collisions between the grains themselves, then there may be focusing into the 'accretion streams' or 'jet streams' considered by some cosmogonists to be essential for the development of the Solar System. If there is a dissipative frictional drag exerted by a diffuse medium in the system, even at a stage when interaction between the grains is unimportant, there may be accumulation of grains in resonant bands in the

gravitational field of the moving planets. Both types of behaviour may be included under the heading of gravitational focusing. The novelty of Gold's discussion is to suggest a crucial cosmogonic role for the second type.

In bald terms the notion is: In the 'early solar nebula' grains were plentiful from direct condensation and from collisions between larger bodies of material. Under the action of the general gravitational field and the 'drag' forces, a grain near the central plane would normally spiral inwards or outwards from the Sun. Such spiralling could, however, be halted or speeded up if the motion became resonant with that of an orbiting planet. Thus lanes of accumulation or avoidance would have been formed. Here Gold remarks in passing that a lane of accumulation could be favourable for 'snowballing'; he suggests this as an explanation of the asteroids in Trojan orbits. He remarks too that in regard to any particular lane at any particular epoch there could be selection with respect to grain size or grain composition.

The main suggestion is that, material having been concentrated into a well-defined lane by resonance effects, when the resonance was broken, such material continued in a well-defined stream though once more in spiral motion; further, when it encountered a planet, there was a significant probability of its being captured into a ring round the planet. There would have been numerous cases of this happening throughout the system. Gold sees no serious difficulty in the passage from grains in circumplanetary rings to larger bodies, and the subsequent coalescence of these into one or more satellites round a planet as we now know them.

The interest of Gold's essay, one ventures to think, is its demonstration that the problem of the origin of satellites is still wide open. It was originally presented at a colloquium. It can scarcely have been intended to do more than throw out suggestions, for the treatment is non-quantitative throughout. This makes it difficult to comment further; however, when Gold writes, 'the step from circumplanetary rings . . . to the formation of satellite bodies is not a difficult one and has been discussed on many occasions' one is bound to remark that although this has indeed been discussed often enough, one is not aware that it has ever been established that satellite bodies must form in these circumstances. Also, although there is much attractiveness in Gold's implication that all the regular satellites might have been formed by one and the same general process, the very great differences between the properties of the main satellites and the minor (regular)

satellites in the actual Solar System give strong indications of the operation of two distinct mechanisms of formation. Maybe we can look forward to a quantitative account of Gold's ideas in a form that could be more critically compared with observation. □

Nutrient balance during succession

from Peter D. Moore

THE developmental growth of an ecosystem, commonly termed succession, involves a steadily increasing biomass, reaching a maximum at the climax, or steady state, which is ultimately attained. The increase in living material during succession places a demand upon the inorganic, abiotic component of the system for those elements necessary for the construction of living tissues. Ultimately the biotic component thus becomes an important reservoir of nutrients—a feature which may contribute very considerably to the overall nutrient capital of an ecosystem (see *Nature*, **254**, 184: 1975). Actual data concerning the changing nutrient demand of the biotic component of an ecosystem during its development are, however, sadly lacking.

Experimental manipulation of watersheds in the Hubbard Brook Experimental Forest in New Hampshire, has provided a certain amount of information on which to speculate. Six small, undisturbed watersheds at this site have been studied intensively over a number of years and data concerning nutrient input from rainfall and output from stream drainage has been collected. In 1965 one of the forested watersheds (15.6 ha in area) was clear-felled and the resulting changes in the chemistry of the runoff waters were subsequently reported by Bormann, Likens, Fisher and Pierce (*Science*, **159**, 882: 1968). One of the most striking changes observed was the increased loss of nitrate nitrogen from the ecosystem following felling; a loss of 1.5 kg N ha⁻¹ yr⁻¹ occurred in a control forested watershed, whilst 58.1 kg N ha⁻¹ yr⁻¹ were lost from the clear-felled watershed. Many cations also increased in concentration in the runoff water, thus permitting the conclusion that disturbance led to increased leaching and therefore a reduced capacity for nutrient conservation.

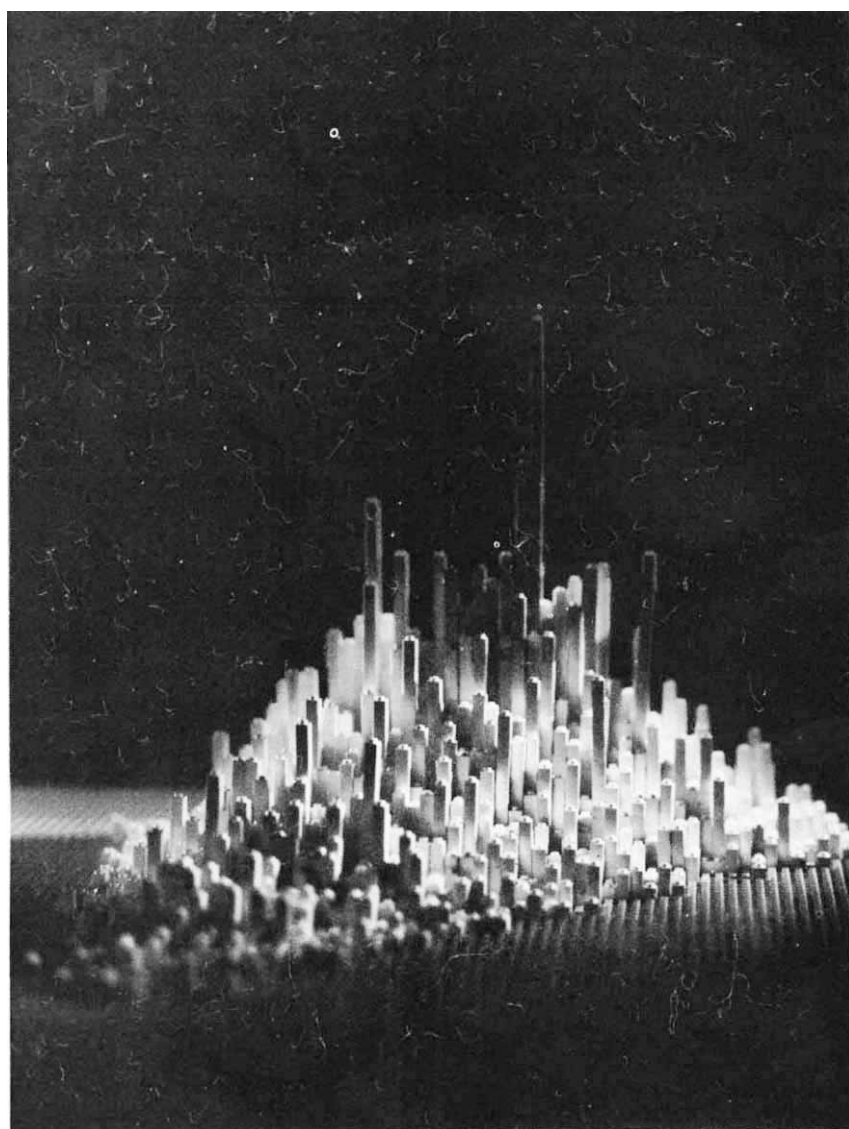
Marks and Bormann (*Science*, **176**, 914: 1972) further demonstrated that revegetation of a disturbed area led to considerably diminished nutrient runoff and they concluded that the early stages of succession which were then

occurring placed a high nutrient demand upon the abiotic environment as the biomass was in a rapid growth phase.

A rather different approach to the problem of documenting the relationship between the stage of ecosystem development and its ability to retain nutrients has recently been made by Leak and Martin (*USDA For. Serv. Res. Note*, NE-211; 1975). They have examined six forested watersheds in the north-eastern United States and have attempted to determine the approximate time when they were last disturbed. Obviously this is a complex problem, since different stands of trees within a stream catchment will have had different histories. A number of stands (between 7 and 41) were sampled within each catchment and such characteristics as the evenness of age within the stand and the proportion of tree species normally favoured by disturbances were noted. In this way it was possible to rank the six sites according to their 'weighted average age'. The time which had passed since general disturbance within the study sites was found to vary between 65 and 200 years.

Streamwater concentrations of nitrate from these watersheds had previously been monitored and these bear an evident relationship to the age since cutting. The site with an average tree age of 65 yr had stream nitrate concentrations of 2.3 p.p.m. (compare recently clearcut areas with 10.3 p.p.m.). Sites with age 75–110 yr trees had 0.15 p.p.m. nitrate in the streamwater, those of 125 yr had 0.8 p.p.m. and those of 200 yr, 2.2–4.8 p.p.m. Similar results have apparently been obtained by Vitousek and Reiners (*Bioscience*, in the press) at Mt Moosilauke.

The evident implication of these data is that low nutrient discharge is a feature of relatively immature ecosystems and that these systems become more leaky on attaining maturity. Nitrogen is a particularly complex element to choose for this type of survey. Not only is its input to an ecosystem complicated by biological fixation, but its output occurs as denitrification and ammonia flux to the atmosphere (see Denmead, Simpson and Freney, *Science*, **185**, 609; 1974) as well as by stream discharge. It is reasonable to assume, however, that as the steady-state ecosystem is approached, the total inputs of the element should be equalled by outputs and this equilibration may well account for Leak and Martin's observed increase in stream nitrate discharge with stand maturity. We may have to wait for a couple of centuries before these suggestions can be confirmed by the controlled experimental techniques of the Hubbard Brook team. □



This Manhattan skyline is a three-dimensional representation of the data from a recent search for charmed particles. A 3.6 GeV/c antiproton beam was used to investigate the interaction $\bar{p}p \rightarrow \bar{K}^0 K^+ \pi^+ \pi^- \pi^0$ and blocks are mounted vertically to count mass values of $(\bar{K}^0 \pi^+ \pi^0)$ against the mass

values of $(K^+ \pi^-)$ of which there are two possible combinations giving the x and y horizontal axes. Peaks are evidence for the existence of charmed particles but those that are seen in these results do not tower sufficiently above their surroundings to be convincing—*CERN Courier* (**15**, 1975).

Solar flares and weather

from John Gribbin

In *Nature* recently Olson *et al.* (**257**, 113; 1975) summarised evidence for a link between solar flares and changes in both the geomagnetic index and atmospheric vorticity on Earth. They ended their paper by saying that "we should examine the position of the flare on the disk of the Sun as a factor" in determining the response of weather systems to specific solar events, and in this context a brief communication just published in *Astrophys. space Sci.*, **35**,

L33–L34; 1975, is of topical interest.

In that paper J-T. Horng (Radio Physics Group, Telecommunications Laboratory, Chung-Li, Taiwan) reports a study of the association of geomagnetic events with complex sunspot groups, covering the period 1968–1972. Horng defines a complex sunspot group as one with two or more spots occurring in one penumbra, and has looked only at large geomagnetic disturbances ($A_p \geq 28$). The data include 36 such disturbances associated with 62 visible complete spot groups, and three geomagnetic events for which bad weather prevented any sunspot observations being made. It emerges that there is a

definite association between complex spots which occur between 6.7 and 19.9°E on the solar disk and the occurrence of large geomagnetic disturbances on Earth. The mean displacement of these spot groups, 13.3°E, can be taken as indicating that the spot groups and flares which cause the geomagnetic disturbances are located one day East of the solar meridian, allowing for their usual movement from East to West. For this particular sample, Horng also points out that 59.7% of the events occurred in the solar Northern Hemisphere below latitude 29°N and 40.3% in the Southern Hemisphere below latitude 25°S. But that is hardly surprising since the entire period of the study was close to the most recent solar maximum, when sunspots always form at low latitudes. □

Image processing at Stanford

from Albert Macovski

A meeting on "Image Processing for 2-D and 3-D Reconstruction from Projections: Theory and Practice in Medicine and the Physical Sciences", sponsored by Stanford University Institute for Electronics in Medicine and the Optical Society of America was held at Stanford University on August 4-7.

DUE primarily to the phenomenal success of the EMI brain scanner, the general subject of reconstruction from projections has generated a great deal of interest and enthusiasm. This particular meeting at Stanford, California is the second one of its type, the first taking place last year at the Brookhaven Laboratories.

Although X-ray and γ -ray imaging continue to dominate the scene, a number of novel application areas were presented which use comparable techniques. T. F. Budinger *et al.* (Lawrence Berkeley Laboratory) showed some preliminary results using heavy charged particles rather than X rays. Although the instrumentation is quite extensive, a significant dose reduction is obtained. J. F. Claerbout and P. S. Schultz (Stanford University) showed the application of similar techniques for seismic imaging using the reflections from sonic pulses. A cross-sectional image of the Earth is thus created to aid in activities such as oil exploration. J. F. Greenleaf, *et al.* (Mayo Foundation, Rochester) showed some exciting images of an excised dog's heart made by measuring the time-of-flight of ultrasonic pulses taken at many angles and positions. The reconstructed image

is a cross section representing sonic velocity. A number of other interesting papers were presented on non-radiographic applications including nuclear magnetic resonance zengmatography, electron microscopy, radioastronomy and plasma diagnosis.

Many papers were devoted to the mathematics of reconstruction, both basic and related to specific applications. There was a strong trend, compared with the Brookhaven Conference, toward the use of direct as compared with iterative reconstruction procedures. In the basic area, R. P. Tewarson (SUNY, Stony Brook, New York) delighted the audience with his very profound fundamental treatment, well sprinkled with humour. He concentrated on methods for reconstructing the difference between the desired image and a standard image which he alluded to as the "average caucasian face." G. T. Herman *et al.* (SUNY, Buffalo), one of the pioneers of the iterative techniques, showed how these techniques converge to the least squares solution. Similarly P. R. Smith (Universitat Basel) along with T. M. Peters *et al.* (Christchurch Hospital, New Zealand) presented a fundamental exposition of the Fourier and back projection methods and the various aliasing considerations. M. Ein-Gal *et al.* (Stanford University) presented a decomposition of the projection data into consistent and inconsistent data and defined a quality factor representing their ratio. This talk invoked a lively discussion between the author, G. T. Herman and R. B. Marr of the Brookhaven Laboratory on the definition of inconsistency. O. J. Treliak (Drexel Institute) presented an analysis of the point-spread function for direct reconstruction systems. This helped explain many of the artefacts which are being experienced.

In the area of mathematics related to specific applications a few investigators, including S. A. Johnson *et al.* (Mayo Foundation) discussed the problem of bending of rays due to refraction when reconstructions are made using optical or sonic waves. R. Gordon (National Institutes of Health), a pioneer in this field and the organiser of the meeting, presented a novel reconstruction system related to position reconstruction systems. Each event, as received, is assigned its most probable spatial position based on the previous events. A number of papers were presented on optimum reconstructions in the presence of incomplete data. L. R. D'Addario of the National Radio Astronomy Observatory and S. J. Wernecke (Stanford University) each presented papers on maximum entropy reconstructions. These gave impressive results in applications such as radioastronomy and electron microscopy,

where the acquired data are sparse. A novel Fourier reconstruction system for nuclear medicine was presented by L. T. Chang *et al.* (Lawrence Berkeley Laboratory). A sequence of pinhole images were back projected and filtered in Fourier space to provide the desired reconstruction.

In the areas of improved X-ray and γ -ray reconstructions, the positron ring camera using coincidence techniques appeared quite exciting. L. Eriksson *et al.* (University of California, Los Angeles) and C. J. Thomson *et al.* (Montreal Neurological Institute) showed promising results. A number of papers were given on the problems of polychromatic effects in X-ray reconstruction. A. Macovski *et al.* (Stanford University) illustrated the nature of the distortion and a method of correction. R. E. Alvarez and Macovski (Stanford) showed how the X-ray spectrum could be decomposed so as to delineate density and atomic number effects in the reconstructed image. A similar decomposition based on chemical elements was presented by M. J. Berggren *et al.* (Mayo Foundation). The fan beam configuration which allows simultaneous acquisition of Earth projection received considerable attention. D. P. Boyd (Stanford University) described a fan beam instrument using a xenon detector and P. Stonestrom *et al.* (Stanford University) discussed the scatter problems. The fan beam geometry presents some interesting mathematical reconstruction problems which were treated by R. King (General Electric, Schenectady) and L. Wang and L. H. Cho (University of California, Los Angeles). □

Phonon scattering in solids

from a Correspondent

A conference on "Phonon Scattering in Solids" was held in Nottingham, UK on August 27-30. The proceedings will be published by Plenum Press.

At a good conference one finds out where the ragged ends in our knowledge lie. The conference recently held in Nottingham was no exception as contradictions were exposed by the close proximity of presented papers, and private conversations removed some of the gloss from published articles. But there were plenty of good new experiments reported which stimulated a lot of thought and the new techniques presented make it now possible to work at higher frequencies and with better spectral resolution.

Spectroscopy using phonons rapidly developed from the days of specific heat and simple thermal conductivity measurements when it became clear that there are resonant scattering processes which scatter phonons of well defined frequencies. Systems such as some magnetic ions in crystals, low-lying states in amorphous materials, copper pairs in superconductors, and charge carriers in semiconductors are strongly coupled to phonons and in many cases can only be studied by their interaction with injected phonons.

The two subjects which generated most enthusiastic discussion were predictably the Kapitza problem and amorphous materials. Heat transfer between two different solids, one of which at least is electrically insulating to stop electronic conduction, can be quite reasonably explained in terms of phonons propagating through the interface according to the classical ideas of elastic waves at interfaces as long as it is recognised that the material at the boundary may be somewhat different to the bulk. The way heat transfers from a solid to liquid or solid helium still remains a mystery however, even though the problem is being tackled with a variety of penetrating experiments. There is now little doubt that some of the phonons ($\sim 10\%$) go from the solid to the helium according to continuum theory so that $q_{||}$ is conserved and that the majority of the energy is carried by phonons which inelastically scatter at the interface. It is also clear that these inelastic processes are associated with the first few atomic layers of helium but the nature of these interface states is only speculative. Candidates such as surface waves, desorbed atoms and low energy rotons were hinted at but there was an understandable reluctance for necks to be stuck out too far after 34 years of looking for an answer.

Amorphous materials are in much the same state. Very elegant experiments were reported which indicate that there is a two- or three-level system which is strongly coupled to phonons and photons but whose nature is unknown. This situation is commonplace in high energy physics but in the solid state it is rare and so emphasises how sophisticated our knowledge of crystals really is. It is apparent that the amorphous states have a whole spectrum of relaxation times both to the lattice and to each other. In essentially d.c. experiments such as specific heat, all the states can absorb energy while in dynamic experiments with ultrasound and thermal conductivity only the states strongly coupled to the lattice phonons are seen. Even these states may not be seen if their relaxation time is too long or the ultrasound power too high, as they become saturated and then not

able to scatter. This last effect has been used to measure the time for interchange of energy between states by hole-burning techniques with ultrasound in a similar way to that used in electron paramagnetic resonance for inhomogeneously broadened lines.

A great deal of work is going into magnetic ions which are strongly coupled to the lattice. Many mysteries remain here but one gets the feeling that the subject is bedevilled by samples. There are always too many resonances than can be accounted for but this is not surprising as very dilute impurities can have a large effect and strains as small as 10^{-6} can shift the energy levels. Even the strains due to the dopant being studied are a problem. When positive identifications can be made however, theory seems largely able to cope.

Phonons from superconducting tunnel junctions are being used increasingly to study all those systems with frequencies up to 1 THz. Frequencies an order of magnitude higher were claimed to be generated by shining an infrared laser on to quartz, however these phonons were not measured directly as they were detected bolometrically. It is not clear why this method works but no doubt much more work will be done on this system. Optical Brillouin and X-ray scattering are also being used in complicated experiments to study phonons.

The many other topics discussed at the conference can be read about in the proceedings which it is hoped will be published by the end of the year. There were some 110 papers which indicates the growing enthusiasm for this subject. $\square$

European astronomy at Leicester

from A. C. Fabian

The first European Conference on Astronomy was held at Leicester, UK on August 12-14.

THE first European Conference on Astronomy recently attracted about 250 astronomers to the hot lecture theatres of Leicester University. At least four orbiting satellites carry European X-ray instrumentation and these provided many of the new results. Radioastronomy was well represented, but there seemed surprisingly little from optical observers.

Transient X-ray sources are the most exciting objects to have been discovered by the Ariel V satellite. Several had been known before the launch of Ariel V last year, but few could have

predicted the large number of bright ones that have now been observed. Estimates seem to indicate one bright transient every 10 days or so, and K. A. Pounds' group at Leicester gave day by day reports on the latest (A0621-00), which is now the brightest X-ray object in the sky. It was speculated that this new source is similar to the binary X-ray sources, and may be associated with accretion on to a white dwarf, neutron star or black hole. This implies a distance less than a kiloparsec or so if the object is not to exceed the Eddington limit for a solar mass object. Above this limit radiation pressure will seriously modify the accretion flow and the result would probably prevent detection of the source. Extrapolation from known X-ray sources would suggest that the optical companion should be visible between about eighth and twelfth magnitude, at most. A better position, to an accuracy of a few arc min should be available and allow for an identification now that the modulation collimators on Ariel V and the American SAS 3 have been pointed in that direction. Spectral changes show a softening of the spectrum as the intensity increased. H. Bradt (Massachusetts Institute of Technology) reported that no periodicities between 0.8 s and 2,000 s were detectable in preliminary data from SAS 3.

Pulsations in the minute range are well known since the discovery of Ariel 1118-61 last December, as recalled by J. G. Ives (Mullard Space Science Laboratory, University College, London). Bradt showed the X-ray "light" curve of A0536+26, a transient source discovered by Ariel V earlier this year near the Crab nebula. A regular dip every 104 s at high energies (>10 keV) degenerates into about six structured dips below about 5 keV. Pulsations of the more constant source, Cen X-3, were shown by I. R. Tuohy (MSSL). He finds a broad double-peaked pulse from the Ariel V data unlike the single pulse that was usually observed from Uhuru. Observations of pulses from Cyg X-1 were reported by G. Auriemma (Frascati). This is remarkable because the pulses, with periods of about 0.083 s were found optically. They represent a significant fraction ($\sim 5\%$) of the optical flux from the system. Only a non-thermal mechanism can radiate so much energy from so small a region.

The recent series of occultations of the Crab nebula, which contains a 33 ms pulsar and was the site of SN1054, have been well covered by X-ray detectors carried on balloons and rockets. F. D. Seward (Lawrence Livermore Laboratory) showed the results of a rocket observation made last November. The moon started occulting the X-ray source soon after

the detectors were locked on, and the signal gradually decayed over the next 150 s or so. Near the end of the flight the detectors were displaced about 10° to take a background reading. A significant drop in count rate was immediately recorded. Seward and his colleagues suggest that this was due to a diffuse source associated with the unocculted part of the nebula. At a temperature $KT \sim 0.8$ keV and luminosity $\sim 10^{36}$ erg s $^{-1}$, this puts at least part of the Crab in line with other X-ray supernova remnants.

Very long baseline interferometry radio observations of the nucleus of NGC1275 (3C84) by E. Preuss (Bonn) and his colleagues contrasted well with the maps shown by A. G. Willis (Leiden) of the large radio galaxies observed from Westerbork. The VLBI observations could be interpreted in terms of a stable map showing 10 components on the scale of ~ 1 milliarc second! (This is about the angle subtended by a man on the moon.) At the other extreme, the large scale emission regions of 3C236 and DA240 could accommodate many Cygnus-As. A compact, highly polarised, and unresolved source has been found at the leading edge of the eastern component of 3C236 which, if not a background source, will be of interest in discriminating between the many theories of radio galaxies.

Many more new and unpublished results and ideas were presented at this very successful conference. In his review of the theory of supernova remnants, S. Gull (Cambridge), showed a memorable radio "photograph" of the supernova remnant Cas A "taken" with the 5 km telescope. The turbulent detail in the shell of this remnant is as dramatic as any optical picture of the Orion nebula. Popular books will never look the same when these new pictures start replacing the old favourites.

The meeting showed that European astronomy is in a healthy state and it is to be hoped that the European Conference on Astronomy may become an annual event. $\square$

X rays and ion transport in liquids

from Andrew Holmes-Siedle

THE use of photographic film to record X rays has always had the disadvantage that one has to wait to see the image. Another less obvious disadvantage is the "chemical fogging" which impedes perception of fine detail. Direct viewing of fluorescent screens demands high doses to the subject or the use of intensifying systems which, though

costly, do not always give enough detail and do not yield a record of the image in "hard copy". There is now growing interest in a method which seems to combine the virtues of each system with cheapness and simplicity and involves some interesting physics. At least two groups are working on it, using the names 'electron radiography' in the USA and 'ionography' in the UK.

The X-ray shadowgraph image is converted into a charge image on a dielectric by the ionisation of a gas or liquid in a chamber and the transport of the positive ions in a straight line to one wall of that chamber, where they collect as stored charge on a dielectric sheet. This charge image can then be developed with a powder in the same way as in the well-known xerographic processes. The transport of the ions is effected by a fairly high applied electric field and no pre-charging of the sheet is necessary. Such an idea may sound simple but it is only recently that a method yielding a satisfactory resolution has been developed.

Early attempts to use electrostatic image formation all involved the use of pre-charged insulator surfaces. Even before the use of film was established, within months of the discovery of X rays, Righi (*The Electrician*, 37, 75; 1896) had found that X rays would discharge ebonite and that the pattern could be made visible with powders. The discharging of a selenium plate by X rays was embodied in the original Xerox patents but, despite some investigation in the 1950s, "xeroradiography" has not been widely used. Criscuolo (*Non-Destructive Testing*, 14, 28-36; 1956) then found that the X-ray-induced conductivity of air over a charged insulator surface could be used to produce an image but that resolution was still poor, mainly because of the small fraction of photons colliding with gas near the plate. The step to a good image was made by increasing both the atomic number and density of atoms above the insulator and by replacing the field from the charged insulator by an applied field, then simply inserting an uncharged insulator foil between the field electrodes and allowing it to intercept and trap the charged species which drifted towards it. Two groups realised the efficiency of this method at about the same time. H. E. Johns, J. W. Boag and others reported favourable results in 1974 (*Br. J. Radiol.*, 47, 519-529) while A. P. Proudian and others presented a verbal description in 1972 and published results in 1974 (*Radiology*, 110, 667-671).

In both of these publications, the density of atoms near the insulator was increased by using a gas at pressure. Xenon at ten atmospheres proved ideal, since this element has an absorption

peak which nicely matches the white radiation spectrum emitted from a 100-keV X-ray tube. The polarity of field is normally such that the xenon ions bombard and charge the surface of the insulator foil. While the electrons will also produce a charge image, the British workers showed that scattering on the way to the foil caused serious loss of resolution. This team thus used the name ionography for the technique, while the American team preferred to call it electron radiography to avoid the historical associations of the former term.

The American team, Allan *et al.*, have now published (*J. appl. Phys.*, 46, 2766-2767; 1975) details of an improved process, termed "liquid-absorber electron radiography", which avoids the use of gas at high pressure. The extra simplicity of using a liquid transporter medium clearly confers engineering advantages but physicists should also be intrigued by this new requirement for efficient transport of charge in a condensed phase. The composition of the liquid will have to be tailored to meet several unusual demands. Not only must it be a good X-ray absorber but its nature must be such as to minimise recombination and scattering of the ions as they drift to the foil. Of course, in order to reduce lateral charge movement and "fog" from leakage between the field plates, the liquid must also be an excellent insulator when not being irradiated. Since it is desirable to sweep out the ions rapidly, fields near to those producing avalanche effects in the medium will be favoured. Since the science of charge transport in normally non-conducting materials in the condensed phase is still in its infancy, this new demand for know-how may give a welcome increase of interest in the basic processes of transport.

The prediction of the British workers, that a very thin pencil of X rays will produce a spot image of radius about 0.175 mm at half maximum in high-pressure xenon is nicely borne out in their experiments, which indicate an image resolution of about 10 line pairs per mm. The results quoted by the US workers for the liquid medium show a strong improvement on this result, the resolution for the latter medium being much better than 10 line pairs per mm. Clearly, charge image radiography has come of age but should still provide some interesting physical problems during its further maturation. $\square$

Erratum

In the article 'Jupiter XIII' (*Nature*, 257, 178; 1975) para 2, line 20 should read "magnitude about +22" not "magnitude about +0.22".

articles

A subharmonic lunar tide in the seas off Western Europe

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A small and often neglected tidal component, which is an exact subharmonic of the M_2 tide, is shown to have unusually large amplitudes over a sea area stretching from Shetland to the Azores, including the North Sea. An explanation is offered in terms of normal oceanic modes recently computed by Platzman, and implications for tidal prediction procedures are discussed.

THE usual picture of the lunar tide-generating potential, a symmetrical oval shape, concentric with the Earth and with its axis directed towards the Moon, is only approximately correct. Because of the finite distance of the Moon the oval is not quite symmetrical, in that the potential on the part of the Earth's surface nearest the Moon is about 1% less than that on the part farthest away. The asymmetry, illustrated in Fig. 1a, produces a daily tidal component which is an exact subharmonic of the principal lunar half-daily tide (M_2), the period of its cycle being a mean lunar day (I ignore the ter-diurnal distortion M_3). This tide is distinct from that normally thought of as the "diurnal tide", and which is due to the monthly excursions of the Moon above and below the Equator. That diurnal tide does not contain a component which is an exact subharmonic of M_2 . If the orbit of the Moon were permanently in the equatorial plane, as in Fig. 1a, there would be no diurnal tides of the usual sort (Fig. 1b), but the subharmonic lunar tide would remain.

The facts set out above are known in tidal theory, but the subharmonic effect is usually neglected in practice because the asymmetry in the generating potential is so small. What is not generally known is that at a number of places, representative of a wide sea area off western Europe, the subharmonic tide actually dominates that part of the spectrum known loosely as the " M_1 tidal constituent". The M_1 group of lines, as it is more properly called, consists of a cluster of harmonic components of the tide-generating potential within 1 or 2 cycles yr^{-1} of 1 cycle per lunar day. It may be split into two distinct groups, one associated with the function

$$\xi^2 P_2^1(\theta) = \xi^2 \frac{3}{2} \sin 2\theta$$

where ξ is the Moon's parallax, and θ is terrestrial colatitude, and the other with

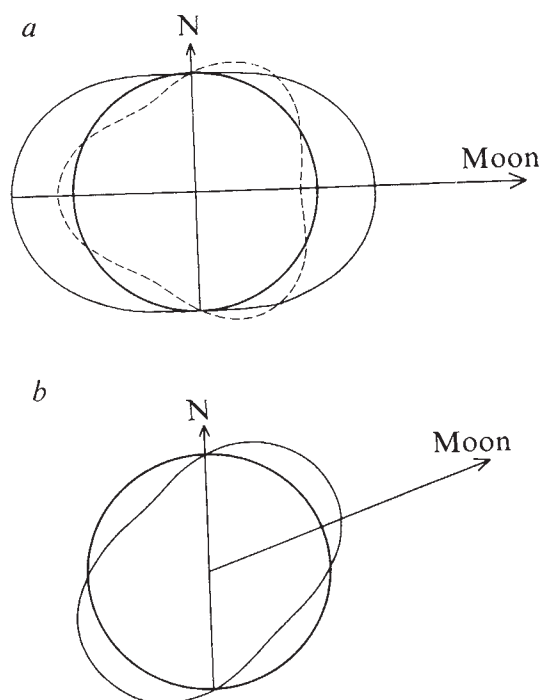
$$\xi^3 P_3^1(\theta) = \xi^3 \frac{3}{2} \sin \theta [(5 \cos^2 \theta) - 1]$$

(P_2^1 is represented in Fig. 1b, and P_3^1 as the broken curve in Fig. 1a). Their spectra are shown in Fig. 2a and b. Since ξ is of

the order 1/60, the second group tends to have much smaller amplitudes than the first, and this is evident when comparing the logarithmic scales of their spectra. The other distinction is that the P_2^1 group is dominated by two lines at about 1/9 cycles yr^{-1} on either side of the central frequency 1 cycle per lunar day whereas the P_3^1 group has essentially one strong line at just the central frequency. Figure 2c, d and e shows the corresponding part of the spectra of 18-yr sections of tide gauge records from Newlyn (two sections) and Brest. Lines corresponding to the M_1 group in the potential show up clearly above noise level, and the salient fact is that the 1 cycle per lunar day (subharmonic) line from P_3^1 is the largest of all at both places. This represents a magnification of the P_3^1 lines relative to the P_2^1 lines by a factor of more than 10.

A similar phenomenon had been observed¹ in the analysis of records from Simons Bay, South Africa covering nine years, but there the P_3^1 line dominated on account of an unusual node

Fig. 1 a, Cross section of components of gravitational potential with Moon over the Equator. —, M_2 component. - - -, Subharmonic M_1 component (magnified $\times 50$ relative to M_2). **b**, Form of principal diurnal component with Moon north of the Equator. In both cases, the circle represents the Earth's surface, and other curves are drawn relative to it.



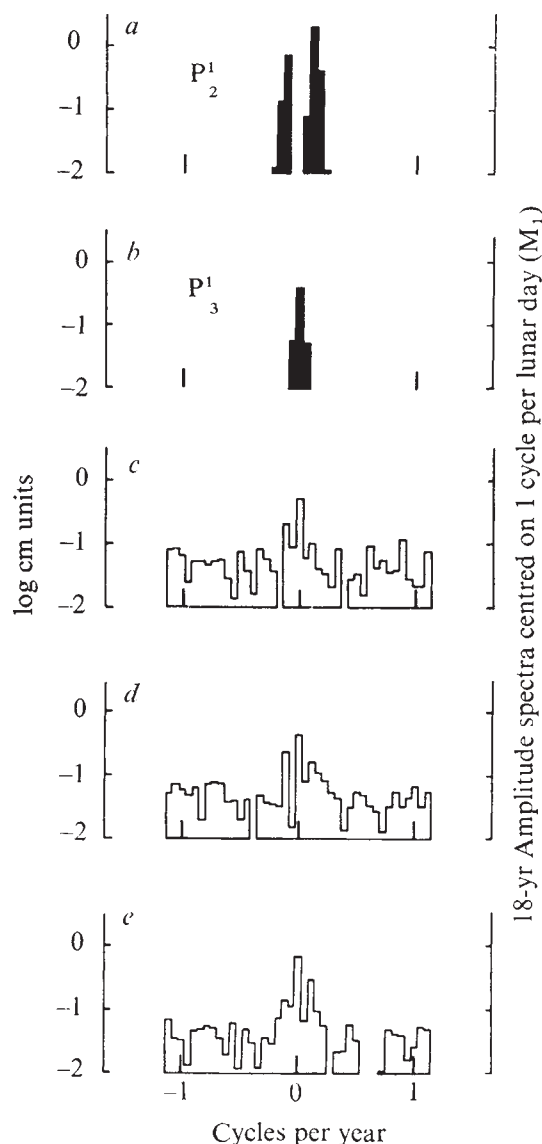


Fig. 2 Amplitude spectra at $1/18$ cycles yr^{-1} resolution near 1 cycle per lunar day. The base scale shows the heterodyned frequency. *a*, P_2^1 gravitational potential; *b*, P_3^1 gravitational potential; *c*, sea level at Newlyn, 1915-33; *d*, sea level at Newlyn, 1951-69; *e*, sea level at Brest, 1915-33.

in the principal diurnal tide at a frequency very close to 1 cycle per lunar day. From further enquiries, it seemed that the subharmonic M_1 tide, as we may call the principal component associated with the P_3^1 harmonic, was first identified in sea level records from Cuxhaven, around 1968 (W. Horn, personal communication). A phenomenon as widespread as Brest, Newlyn and Cuxhaven suggests an oceanic origin, so I searched for other European sea level records of sufficient length for its resolution. The line-separation of $1/9$ cycles yr^{-1} requires 9 yr of records for clear resolution, but 5-6 yr are adequate with special methods², if the noise level is low. Records from Southend covering 18.5 yr recently analysed for other objectives (M. Amin, to be published), was found to have a subharmonic M_1 tide similar to Cuxhaven. Shorter records from the Scilly Isles, Malin Head, Stornoway and Lerwick confirmed its dominance, as at Newlyn, over the entire oceanic coastline of Britain. Another short record, from Dunkerque, gave results similar to Southend.

The results from the places mentioned previously are shown in Table 1. H and G are the amplitude (mm) and Greenwich phase lag (degrees) of the named constituents, and R the amplitude ratio (admittance) to the gravitational potential, in the

normalisation of Cartwright and Tayler³. I have used M_1' to denote the subharmonic tide (in accordance with modern German practice⁴), and M_1 the principal component of the P_2^1 tide, which some⁴ prefer to call "NO₁". The principal lunar semidiurnal tide M_2 is included for comparison. The three entries for Newlyn are for three consecutive 18-yr spans of data, the spectra from two of which are included in Fig. 2; they show the order of variability which may be expected due to noise. There is no obvious relationship between the constants of M_1 , M_1' and M_2 , but the main features described above, large H and R for M_1 , small for M_1' , are clearly supported. One notices that the amplitude ratios for M_1 are about 1-2, which is not very large, but nevertheless comparable with the admittances at M_2 which are generally considered 'large' in the north-east Atlantic. The admittances for M_1' are, however, only of the order of 10^{-1} , which makes their H values small in comparison with those of M_1 . The dominance of M_1 in this area, then, is due not only to magnification of the P_3^1 tide but also to a depression of the common diurnal tide in the Atlantic Ocean as a whole.

The two island stations included at the bottom of Table 1 show that at the distance of the Azores, the dominance of M_1 is reduced to equality in H , whereas as far as Bermuda R become of similar order, and M_1' dominates. The result for Bermuda is in fact the norm, confirmed at for example Honolulu (19 yr), Venice (12 yr), and Antarctica (9 yr). The phenomenon of a relatively magnified M_1 may well be confined to western European seas (and the vicinity of Simons Bay).

Heuristic explanation

Before proposing a physical cause for the enhanced M_1 or suppressed M_1' tide, it is worth dismissing some possible but implausible suggestions.

(1) Anomalous lines in tidal spectra are often due to non-linear interactions at sum and difference frequencies. This can indeed affect M_1' , through interaction between N_2 and O_1 , but only noticeably at the shallow water ports of Cuxhaven and Southend. There are no tidal lines of any importance whose frequencies differ by that of M_1 (1 cycle per lunar day).

(2) One might envisage a nonlinear mechanism causing a subharmonic-harmonic instability between M_1 and M_2 . But this would suggest some correlation between the amplitudes of M_1 and M_2 , which is not observed (Table 1). At these places there is nothing anomalous also about the diurnal line K_1 , which is a subharmonic of K_2 .

(3) Some tides are amplified at the resonant frequency of a semi-enclosed basin, as in the Bay of Fundy. But a resonance which selects M_1 and not M_1' implies a tuning width of about 0.1 cycles yr^{-1} ; such fine tuning is unknown in oceanography.

My suggested explanation involves spatial matching of forcing function and normal modes of oscillation, rather than resonance in frequency. Figure 3*a* and *b* shows the wave form of a normal mode of the Atlantic-Indian Ocean system at a period of 23.5 h, computed by Platzman⁵. This is the nearest in frequency to the diurnal tides of many such modes, and can be expected to form an important contribution to the forced tidal wave at 1 cycle per lunar day. Concentrating on the Atlantic, which has the largest wave elevations, one sees an anti-clockwise amphidromic system in mid-northern latitudes and a similar clockwise system in mid-southern latitudes. The arrows indicate the direction of wave propagation at (arbitrarily) 0 hours. At that time in the North Atlantic there is a northbound wave crest with northward currents in the eastern sector, whereas to the west of the amphidrome there is a southbound wave-trough, which also has northwards currents. So at 0 h there are general northward currents in the North Atlantic, and similarly there are general southward currents in the South Atlantic. A quarter of a cycle later there will be generally westward currents in both North and South Atlantic; half a cycle after 0 h, southward currents in the North Atlantic, northward currents in the South Atlantic, and so on. The point is that this pattern of motion matches the rotary motion of the

Table 1 Positions of places analysed, and the amplitudes H (mm), phase lags G (degree), and admittances R of the subharmonic M_1 tide, of the nearby P_3^1 term (M_1'), and of the M_2 tide

Place	Latitude	Longitude	M_1			M_1'			M_2			yr
			H	G	R	H	G	R	H	G	R	
Newlyn, Cornwall I	50° 06'N	05° 33'W	5.3	284	1.3	1.0	39	0.05	1,700	135	2.69	18
Newlyn, Cornwall II	50° 06'N	05° 33'W	4.6	274	1.1	1.1	26	0.05	1,704	135	2.70	18
Newlyn, Cornwall III	50° 06'N	05° 33'W	4.4	277	1.1	1.6	49	0.08	1,702	135	2.69	18
Scilly Isles, Cornwall	49° 55'N	06° 20'W	5.0	276	1.2	3.1	43	0.15	1,764	130	2.79	6.25
Brest, Brittany	48° 23'N	04° 30'W	6.9	268	1.7	3.0	31	0.15	2,401	109	3.23	18
Malin Head, Eire	55° 22'N	07° 20'W	8.9	312	2.2	5.6	101	0.27	1,061	183	1.68	8
Stornoway, Hebrides	58° 12'N	06° 23'W	8.8	308	2.2	6.7	81	0.32	1,363	198	2.16	6.5
Lerwick, Shetland	60° 09'N	01° 08'W	7.4	330	1.9	3.7	91	0.18	585	312	0.92	9
Southend, Essex	51° 31'N	00° 44'E	9.5	112	2.4	2.5	88	0.12	2,045	354	3.23	18.5
Dunkerque, France	51° 03'N	02° 22'E	9.2	104	2.3	6.6	101	0.32	2,103	353	3.30	5.25
Cuxhaven, Germany	53° 51'N	08° 44'E	9.2	214	2.3	(below noise)			1,322	344	2.09	23
Terceira, Azores	38° 40'N	27° 14'W	2.1	275	0.5	2.2	41	0.11	447	65	0.71	5.25
St Georges, Bermuda	32° 24'N	64° 42'W	1.0	260	0.3	3.4	191	0.16	357	358	0.57	9

P_3^1 forcing function, but mis-matches the motion of the P_2^1 forcing function.

The semicircular arrows in Fig. 3c and d represent the azimuth direction of the horizontal stress due to the P_3^1 (left) and P_2^1 (right) components of the Moon's gravitational potential, during a half cycle starting with Greenwich transit (tails of arrows). The direction of rotation reverses across certain latitudes which are drawn. In the wide zones between latitudes 26.5° and 59° the P_3^1 stress rotates in the same sense and phase as the two gyres of the Atlantic in Platzman's model, and with some allowance for change of longitude, the more northwards Indian Ocean gyre is matched also. The matching in the gyre below 60°S is not so good, but on the whole the work done on

this mode of motion by the P_3^1 stress, as measured by the scalar product (stress $\times$ current) integrated over the whole area will be rather high. The P_2^1 stress pattern, on the other hand is much less effective, being northerly in both north and south (below 45°) at 0 h, then westerly in the north Atlantic and easterly in the south, quarter of a cycle later, and so on. The work integral for P_2^1 will be small, and one expects this stress pattern to generate rather weak tides, as observed.

Further support for the importance of Platzman's near-diurnal mode comes from the large amplitudes above latitude 45°N, shown by the numbered broken lines in Fig. 3. The observed amplitudes for M_1 are large in just this area, and do in fact increase towards the north. (The largest amplitudes of

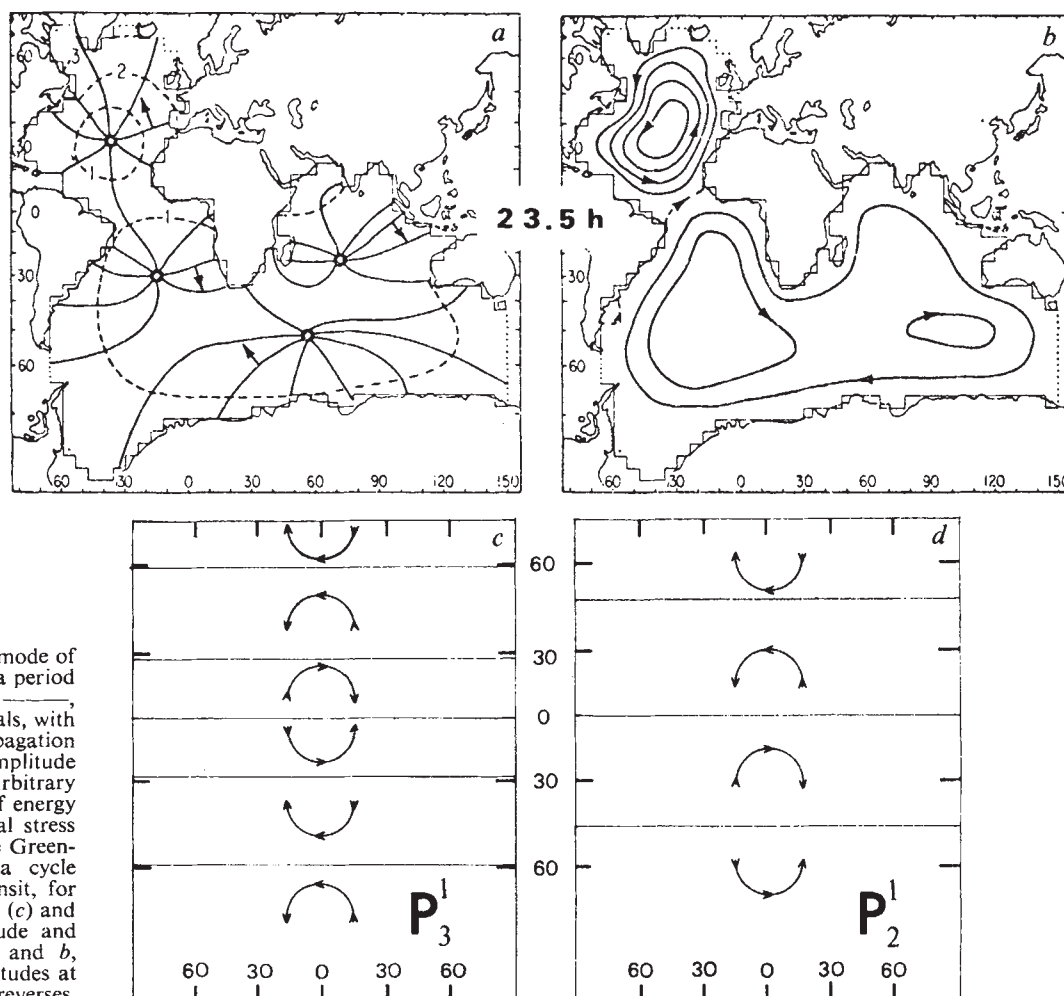


Fig. 3 *a-b*, Gravity wave mode of Atlantic/Indian Oceans at a period of 23.5 h (after ref. 5). *a*, —, isophase lines at 45° intervals, with arrows in direction of propagation at 0°; ----, isoamplitude lines with amplitude in arbitrary units. *b*, stream function of energy flux. *c-d*, Direction of tidal stress (potential gradient), on the Greenwich meridian for half a cycle (180°) following lunar transit, for P_3^1 component of potential (*c*) and P_2^1 component (*d*). Latitude and longitude scales as in *a* and *b*, straight lines mark the latitudes at which sense of rotation reverses.

all, at Cuxhaven and Southend, may be regarded as a displaced product of the tides at the northern entrance to the North sea.) One would expect from Fig. 3 to find still larger amplitudes in north-east Canadian waters, but this has not been investigated. The accuracy of Platzman's model may in any case fall off near its northern boundary.

Implications for tidal prediction

The classical " M_1 tidal constituent" (my M'_1) has always fitted awkwardly into the conventions of "harmonic" prediction, on account of its two major spectral lines separated by 0.23 cycles yr^{-1} , which is too close for resolution from a year's analysis but rather far for an accurate 'slow modulation' scheme. Schureman⁷ devoted three pages to it; Rossiter⁸ included it in a discussion of "troublesome harmonic constituents" and referred to an epistolary dispute about M^1 between Horn and Doodson in 1947. The usual method, which ignores the possible existence of the subharmonic term, takes a nominal frequency of 1 cycle per lunar day ($14^\circ.492 \dots \text{h}^{-1}$) but adjusts with a modulating function whose phase advances from 0 to 360° every 9 yr. Even the definition of the modulating function differs according to authority^{7,8}, so that published constants are valid only if used by the method of the authority which produced them. This odd situation has survived over the years only because the constituent is, after all, rather small, so that errors in prediction arising from it will not be too serious.

The identification of the subharmonic M_1 in the sea area specified confuses the issue still further, and a reappraisal of the whole situation is needed. It is worth mentioning three logical solutions to the difficulty.

(1) One may treat M_1 and the two companion terms of M'_1 as three distinct constituents, each of which has a minor modulating function depending on the lunar 'node', as in common constituents. This is, in fact, the method suggested by the recent DHI tables⁴. To identify these constituents, however, requires several years of data to be analysed, typically 9 yr, or preferably 18 yr.

(2) The "response" method² may be used, which automatically treats P_2^1 , P_3^1 and nonlinear terms separately in an optimum way. This still requires a minimum of 5–6 yr of data to achieve the separation reliably.

(3) If one is restricted to the standard '1-yr analysis' a compromise must be made, depending on the sea area. In areas where the subharmonic M_1 tide has been shown to be normally small, the standard modulation scheme is adequate, although some unification of constants would be desirable. In north-west European seas, I have shown here that one would do best to treat M_1 as a pure subharmonic line, and either ignore M_1 or infer it from continuity with the more reliable terms O_1 and K_1 from the P_2^1 tide.

I am grateful to Mr R J Tayler and Mrs Anne Edden for assistance with the analysis of the Newlyn data.

Received May 20; accepted August 8, 1975.

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Meanders and long waves in the equatorial Atlantic

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Observations from the GATE Equatorial Oceanographic Experiment are presented. They reveal large scale meandering of the westward flowing South Equatorial Current and of the eastward flowing Equatorial Undercurrent with time scales of 2–3 weeks. Meandering of the flow pattern was found to be related to corresponding displacements of the high salinity core of the undercurrent. The observations tend to support the assumption of a long wave propagating westward with a phase speed of 2.3 m s^{-1} and a wavelength of 3,200 km. A possible explanation may be given in terms of unstable waves caused by large scale horizontal shear in the Equatorial Current System.

THE Equatorial Experiment, one of the components of GATE (Atlantic Tropical Experiment of the Global Atmospheric Research Programme, see refs 1–3), was an oceanographic experiment designed to determine the spatial and temporal

scales of transient equatorial phenomena, to study their interaction with equatorial currents, and to investigate the dependence of upper ocean phenomena on atmospheric forcing. The platforms from which measurements were made included stationary and roving ships as well as an array of moored buoys carrying measuring equipment. This experiment culminated during phase II of GATE, from July 27 to August 16, 1974 (see Fig. 1). Complementary observations were made during phase I of GATE, which preceded this project, and phase III, which followed it. Here we report on preliminary results from the Equatorial Experiment and deal mainly with large scale meandering and wave phenomena of the currents within $\pm 50'$ latitude of the equator. Information on these phenomena is based on: (1) current profiling measurements from the research vessels *Capricorne* (France), *A. Dohrn* (FRG), *A. V. Humboldt* (GDR), *C. Iselin* (USA) and *Ak. Kurchatov* (USSR); (2) moored current meter arrays positioned by the research vessels *Discovery* (UK), *A. Dohrn*, *Ak. Kurchatov*, *Passat* (USSR) and *Trident* (USA); (3) measurements of the temperature and salinity fields from all vessels mentioned above as well as additional measurements from the *RV Atlantis II* (USA).

The observational techniques in categories (2) and (3) are usual oceanographic procedures, but the observations in category (1) constitute a novel approach (for details on the

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Fig. 1 Central observational array during the GATE Equatorial Experiment.

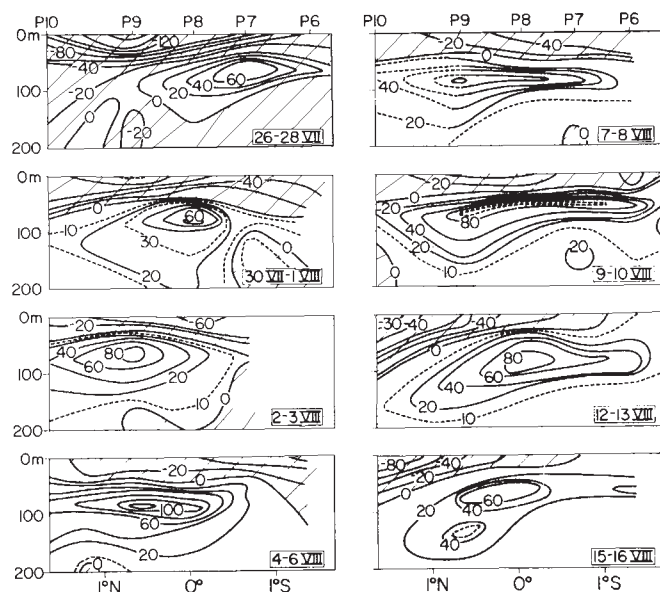
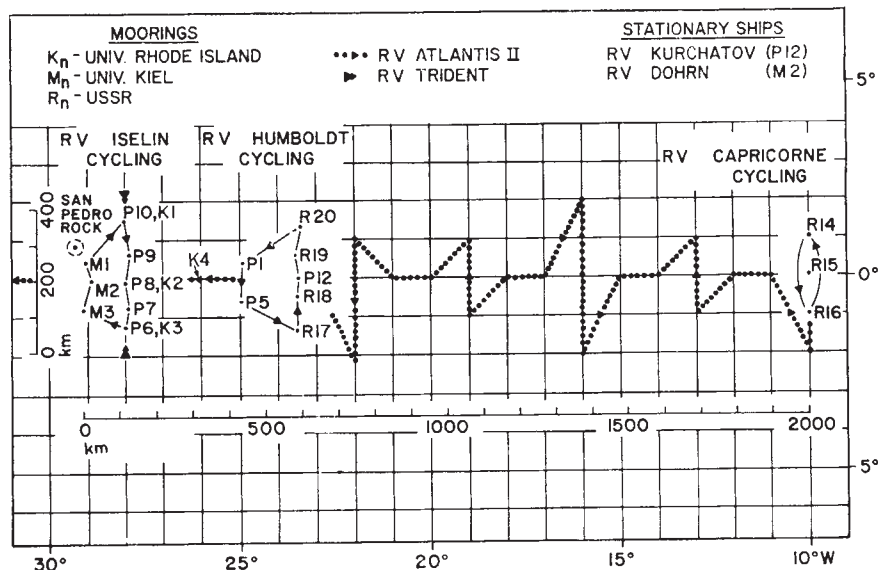


Fig. 2 Consecutive meridional sections of the east-west current component along 28°W observed by RV Iselin. Isotachs are in cm s^{-1} ; hatched areas denote westward flow.

profiling current meters (PCMs) and on their mode of operation see refs 4 and 5). The Humboldt used the SRS method⁵ to obtain complementary observations of current profiles.

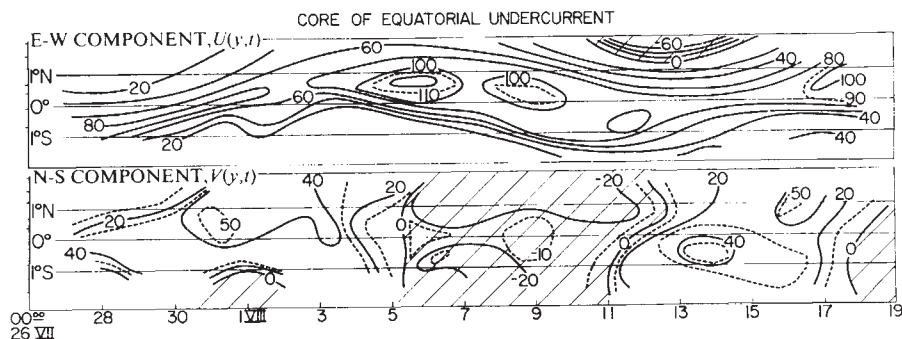
Meandering of equatorial currents

RV Iselin and RV Humboldt operated in cyclic modes along the western and the eastern trapezoids respectively (Fig. 1). This

procedure made possible the quasisyntopic observations that indicated meandering motions of the flow field as well as of the fields of temperature and salinity at all depths from the surface to well below the core of the Equatorial Undercurrent. Consecutive sections from July 26 to August 16 along 28°W show that the eastward-flowing core of the undercurrent meandered approximately between latitudes 0°50'S and 0°50'N (Fig. 2). Similar sections made by RV Humboldt along 23°30'W confirm that the meridional excursion of the core of the undercurrent occurred within ± 50 miles of the equator. Maximum core displacement took place over a period of 1 week at both, 28°W and 23°30'W. Thus, core displacements at both longitudes occurred on time scales much shorter than previously believed. The jet-like core of the undercurrent displays a pulsating behaviour: the volume transport in the core at 28°W, as obtained by integration within the 20 cm s^{-1} contour (Fig. 2), varied over a range from $4 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ on July 27 to $15 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ on August 5. This feature is also shown in the time-latitude sections in Figs. 3 and 4a, for example, by following the 60 cm s^{-1} isotach in the east-west component in Fig. 3. Pinching of the isotachs occurs around July 30 and around August 16; widening of the isotachs, accompanied by pronounced velocity maxima, occurs around August 7 at 28°W and about 3 d earlier at 23°30'W.

The westward surface flow was unusually strong, although intermittent, throughout the observational period. The westward core of the surface current showed meridional excursions similar to those observed at the level of the undercurrent (Fig. 5). The northerly component at position M2 (Fig. 6) indicates that fluctuations at the surface level at 28°W lag approximately 3–4 d behind changes at the undercurrent level. Such a vertical lag does not seem to occur at 23°30'W. Another phenomenon observed only at the western trapezoid was a

Fig. 3 Time-latitude sections of the flow components at the core level of the undercurrent based on all stations along 28°W and 29°W. Maximum eastward value from each profile was selected at depths varying between 60 and 90 m. Observations by RV Iselin.



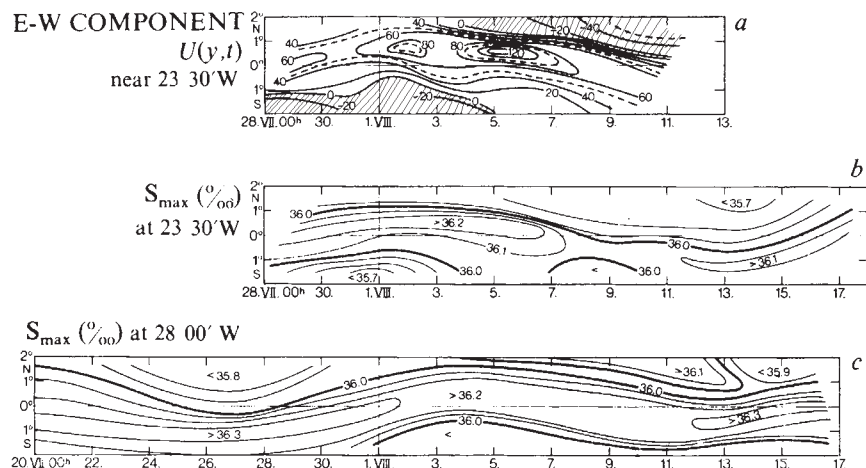


Fig. 4 Time-latitude sections of the east-west current component and of salinity. *a*, Observed by RV Humboldt at 23°30'W; otherwise corresponding to $U(y, t)$ -plot in Fig. 5; *b*, salinity maximum at 23°30'W observed by RV Humboldt; *c*, salinity maximum at 28°W combined from observations by RV Iselin and RV Trident.

surfacing of the undercurrent on August 10 at latitude 1°30'S when weak eastward flow occurred at the surface. By August 15, eastward flow at the surface was observed in the region from 1°30'S to 0°50'S. Remarkably, this happened in spite of prevailing winds from the south-east of about 7 m s^{-1} . This gives the impression that surfacing at 28°W is intrinsically linked to the meandering and wave processes of the equatorial currents.

Comparison of salinity sections (Fig. 4*b, c*) with sections of the eastern component Fig. 4*a* shows that the salinity maximum is a fairly reliable indicator of the meandering of the undercurrent, although the velocity core and the salinity maximum,

data to detect meandering and wave phenomena at other times and locations.

A meander period of about $16 \pm 2 \text{ d}$ is compatible with all observations presently on hand, including those before phase II of GATE. Corroborating observations were made by RV Kurchatov during phase I at a buoy station at 0°, 23°30'W. Bubnov *et al.*⁷ report results from a series of consecutive temperature casts made between June 28 and July 15, 1974. The temperature variability of the thermocline at the depth of the core of the undercurrent shows a pronounced 8-d periodicity. In conjunction with the dome-shaped temperature

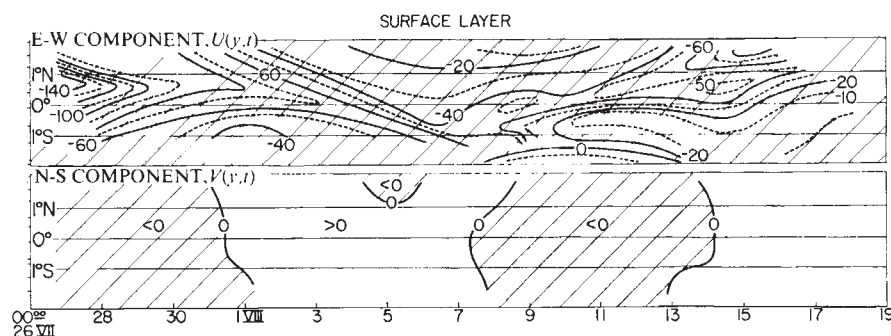


Fig. 5 Time-latitude sections of the flow components at the surface based on all stations along 28°W and 29°W. Values between 0 and 15 m were averaged. Hatched areas denote westward respectively southward flow. Observations by RV Iselin.

in most cases, do not occur at the same depth. Usually the salinity maximum is found above the velocity core in the region of strongest vertical current shear. In spite of these differences in vertical structure, horizontal meandering of the undercurrent may be detected from salinity observations alone. For example, salinity sections made during ICITA⁶ at 8°W indicate meandering of the undercurrent with a half period of approximately 14 d, and with meridional excursions of the core to the north and to the south of the equator similar to the excursions observed further to the west during GATE. There is, however, some evidence from the observations by RV Atlantis II that such correlation does not always exist. If the correlation between the salinity field and the flow turns out to be sufficiently reliable, it may be possible to use historical salinity

distribution at the same longitude between 1°30'N and 1°30'S. Bubnov *et al.* conclude that the undercurrent core meandered across the equator with a 16-d period.

Observations by RV Atlantis II at 28°W also fit this picture. Between July 15 and 17, the eastward core of the undercurrent at 28°W was located approximately 44 miles north of the Equator. The observations by RV Iselin (Fig. 3) show the undercurrent to be at its peak northerly excursion at about July 18–20, one-half period before the start of phase II. Furthermore, RV Atlantis II made 17 sections across the undercurrent from 10°W to 33°W before phase II. These observations establish that the undercurrent was frequently displaced off the Equator and also that meandering occurred within 0°50'N and 0°50'S.

Table 1 Eastward and westward propagating waves

$$\text{Westward propagating waves} \\ c_n = \frac{\omega}{k_n} = \frac{\Delta x}{\Delta T + nT}; n = 0, 1, 2, \dots$$

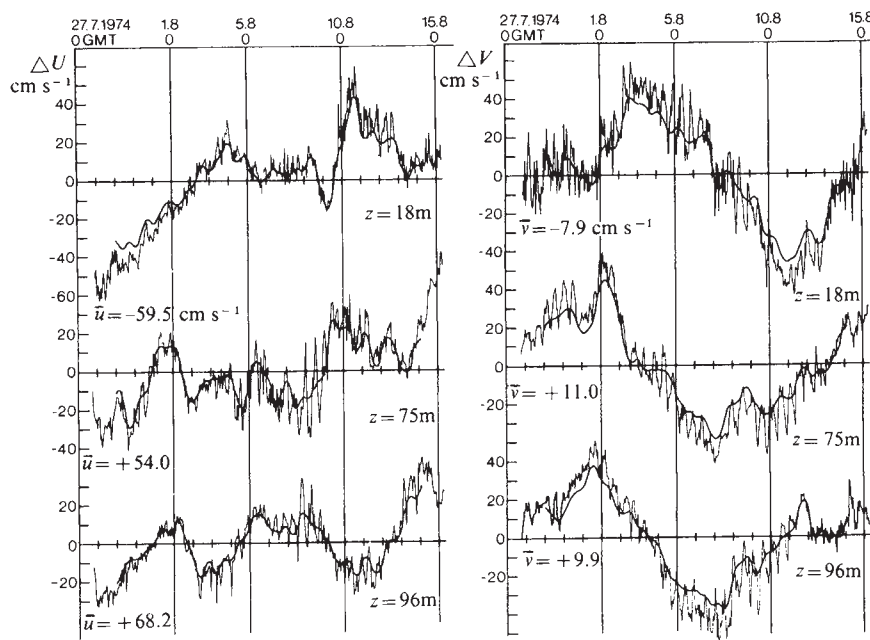
n	$L(\text{km})$	$c(\text{m s}^{-1})$
0	2,660	1.93
1	432	0.31
2	231	0.17

Where $T = 16 \text{ d}$; $\Delta T = 72 \text{ h}$; $\Delta x = 500 \text{ km}$

$$\text{Eastward propagating waves} \\ c_n = \frac{\Delta x}{nT - \Delta T}; n = 1, 2, \dots$$

n	$L(\text{km})$	$c(\text{m s}^{-1})$
1	615	0.45
2	271	0.20

Fig. 6 Flow components in the upper layer observed at three fixed levels at 0°, 29°W (Position M2) from a surface mooring deployed by RV Dohrn. Smooth line obtained from low pass filtering. Amplitude reduction for lower frequencies due to particular filter response.



Fixed level current meter records made from RV Dohrn during phase II confirm the previously mentioned meander time scales (Fig. 6). A particular feature may, however, be mentioned here since it has important ramifications when interpreting records from fixed level meters. Whereas the northward component shows basically the same long periods as those in Figs 3 and 5, the eastward component at the fixed levels at position M2, at 0°, 29°W shows half that period ($\approx 8-9$ d). An apparent doubling with frequency 2ω will occur when a jet-like flow meanders back and forth with frequency ω across a fixed observational point. The representation in Fig 3-5 circumvents this problem.

Wave propagation

A study of the time-latitude distribution of the eastward component at the core level of the undercurrent at 28°W (Fig. 3) shows a pattern that closely resembles the one observed at 23°30'W (Fig. 4a). There are three specific features that are strikingly similar in both observations: (1) long period shifting of the undercurrent core across the Equator, (2) appearance of two cell-like maxima in the eastward component, and (3) development of westward flow north of the Equator towards the end of the observation period. The latter observation is confirmed by observations from RV Kurchatov at position P12 (ref. 8). The time-latitude distributions of the salinity maxima at both longitudes (Fig. 4b, c) are independent of the flow field, also show long period meandering of the salinity core and are in good agreement, even in some smaller scale features.

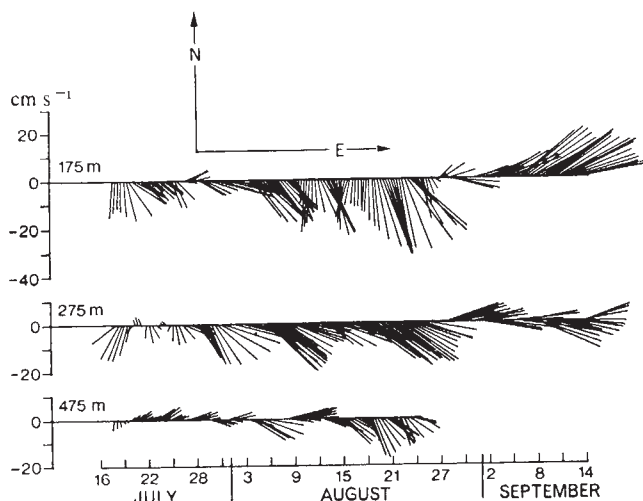
Both flow fields and salinity fields at the two longitudes which are separated by 500 km reveal that the pattern at 28°W lags several days behind that at 23°30'W. Combining all information now available gives a lag estimate of $\Delta T \approx 72 \pm 7$ h. Using this information, simple geometric reasoning gives eastward or westward propagating waves according to Table 1. Thus, there are basically two alternatives: (1) very long westward moving waves, or (2) much shorter, westward or eastward moving waves. It turns out that the PCM observations from the eastern and western trapezoid (Fig. 1) support the assumption of a long westward propagating wave (S. A. Thorpe, personal communication) with $L = 2,600 \pm 390$ km and $c = 1.9 \pm 0.3$ m s⁻¹. Phase relationships between 28°W longitude (positions P6 to P10) and 29°W (positions M1 to M3) longitude were not sufficiently convincing to justify an interpretation in terms of short eastward (or westward) moving waves. A final determination of phase propagation in the various frequency bands is

expected to be forthcoming from current meter records of longer duration.

Observations made by RV Capricorn at 10°W were carried out over a relatively short period, from July 30 until August 10; meridional station spacing was 60 miles, and resolution is therefore limited. A detailed comparison and phase alignment with the observations further to the west is therefore not possible. There is no doubt, however, that large scale meandering also occurs at this longitude. During phase II of GATE, the northward component of the flow was predominantly positive at the level of the undercurrent with values $v \approx 40 \pm 18$ cm s⁻¹, whereas the eastward component was $u = 85 \pm 19$ cm s⁻¹. This is in contrast to a sequence of 18 current profiles observed at 3-hourly intervals at 10°W during August 1973 (ref. 5). At that time the mean value v was southward, $v \approx -42 \pm 12$ cm s⁻¹, at the level of the undercurrent, whereas $u \approx 55 \pm 15$ cm s⁻¹; that is, during both years, 1974 and 1973, the undercurrent veered between 25° and 35° northward or southward respectively from its course due east.

Concluding the observational part, we note that the 16-day wave or meander is the dominant feature in the surface layer

Fig. 7 Low pass filtered current vectors at three fixed levels below the core of the undercurrent at 0°, 28°11'W (Position K2) from a subsurface mooring positioned by RV Trident.



and in the core level of the undercurrent. Additional observations by Meincke⁹, as well as those from a subsurface instrumented mooring positioned by RV Trident, clearly show that a 16-d wave is not a predominant feature at depths below 200 m. As shown in Fig. 7, the predominant oscillations are of both shorter and longer periodicities; of the order of 3–4 d and of the order of 1 month. These oscillations are coherent between 28°W and 26°W.

Probable cause of meanders

Cross-equatorial winds may displace¹¹ the core of the undercurrent from the Equator. An abrupt change in the winds could thus cause the undercurrent to meander across the Equator while it adjusts to the new wind field. It is, however, improbable that the meanders described (here) were generated in this manner because vessels on the equator on all longitudes from 10°W to 29°W found the winds to be remarkably steady throughout GATE.

A preliminary analysis (G. Philander, unpublished) of the stability properties of the equatorial currents reveals that the Equatorial Undercurrent was stable during GATE, but that the surface currents—the westward South Equatorial Current at and south of the equator, and the eastward North Equatorial Countercurrent to the north—had sufficient latitudinal shear to be inertially unstable. The curvature of the earth (β -effect) and equatorial divergence can both destabilise this current

system. The westward propagating unstable waves are predicted to have a wavelength of about 2,000 km, a period between two and three weeks, and an e-folding time of about one month. These numbers and the predicted phase lag between disturbances in the surface layers and those at greater depths are in reasonable agreement with the measurements described earlier.

If the surface currents are indeed unstable then the meander of the undercurrent at the equator is only part of a phenomenon with a much larger latitudinal scale. Furthermore, waves with a wavelength in excess of 2,000 km in an ocean basin which, at the equator, is barely 5,000 km wide, are likely to excite other modes of oscillation. Further analysis of the GATE data should provide answers and could potentially change our present view of the generating mechanism.

Note added in proof: Additional observations supplied by Dr V. A. Bubnov show that oscillations with comparable amplitude and period also occurred during phases I and III of GATE.

Received June 20; accepted August 1, 1975.

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External dose estimates for future inhabitants of Eniwetok Atoll

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Measurements of environmental γ radiation levels on Eniwetok Atoll were combined with pertinent population distributions and expected future life styles to assess the external doses that could be received by the Marshallese people on their return to the former US nuclear weapons test site in the Pacific.

WITH the prospect of habitation in the near future, a comprehensive survey of the total radiological environment of the Eniwetok Atoll was undertaken to provide a basis for determining whether or not the atoll can be safely reinhabited by the former Marshallese population¹. As an integral part of the total radiological survey plan, a study was made to assess the total external dose that the returning population might receive as a result of the radiological contamination distributed in the environs of the atoll. Eniwetok Atoll was one of the US nuclear weapons testing sites in the Pacific, and is situated in the northern part of Micronesia in the Central Pacific Ocean about 3,800 km south-west of Honolulu. The atoll consists of 40 islands on an elliptical coral reef surrounding a lagoon with major and minor axes dimensions of 37 and 27 km, respectively.

The islands, which were given male and female code names during the US occupancy, are shown in Fig. 1. The total land area is about 7 km² with the land height generally averaging 3–5 m above mean sea level. The islands vary in size from small sand bars of a few hundred square metres to heavily vegetated islands of about 1.5 km². The largest islands, and therefore those of most importance for future habitation, are Fred, Elmer and David in the southern part and Janet in the northern part of the atoll.

A total of 43 nuclear tests took place during the 1948–58 testing period. Most of the tests were conducted throughout the northern part of the atoll. Slightly over half of the nuclear devices were detonated over the lagoon and ocean areas whereas the remainder were detonated on several of the northern islands. As a consequence, the major radiological impact of the testing programme occurred throughout the northern islands with only minor radioactive contamination on the southern islands.

Since the external dose is almost entirely the result of γ -emitting radionuclides, with only minor contributions from α and β emitters, it was essential to obtain the best possible description of the geographical variability of the γ exposure rates in air on each island. The following is a brief description

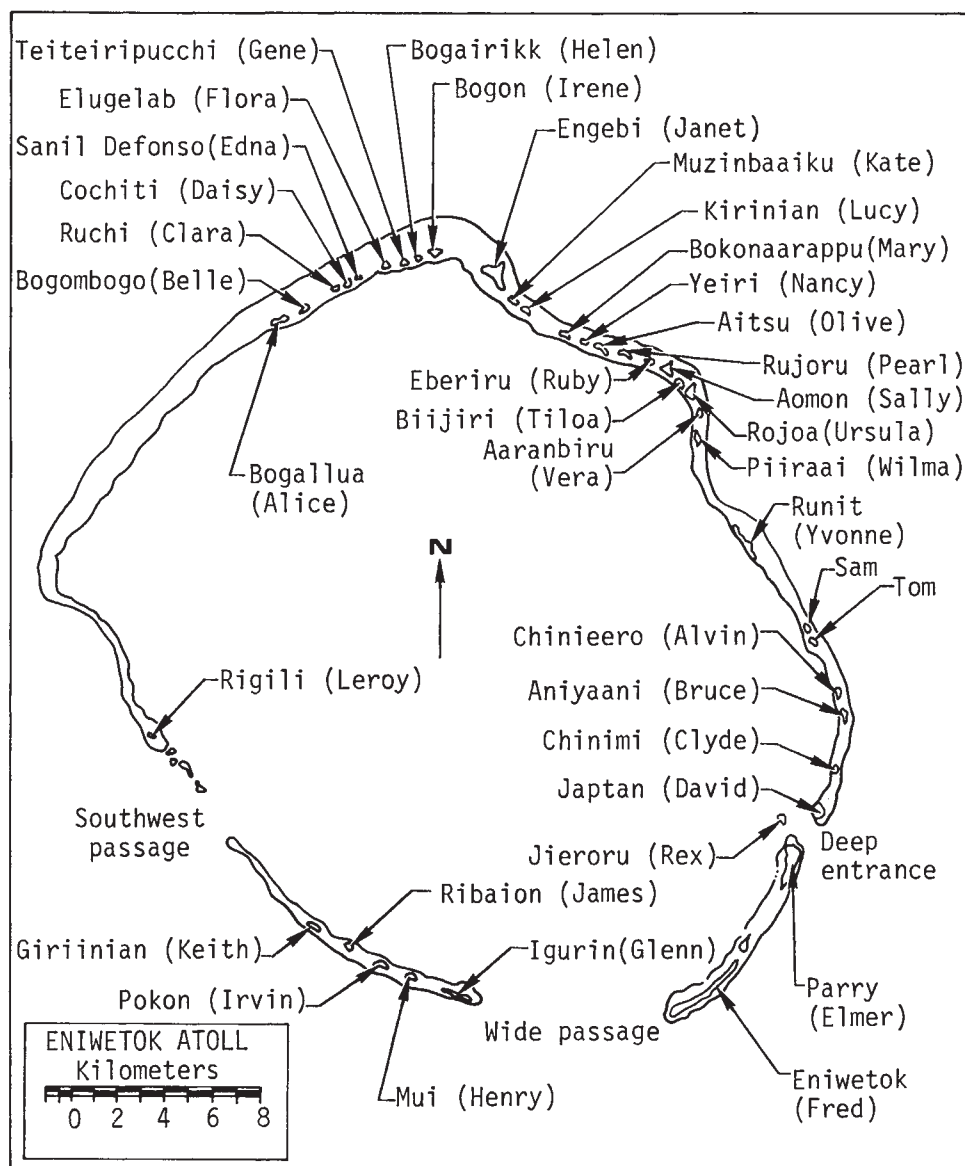


Fig. 1 Map of Eniwetok Atoll.

of the techniques used to measure these exposure rates and how the resulting data, in conjunction with pertinent population statistics and expected life style, enabled us to make realistic estimates of the external dose to future inhabitants.

Measurement techniques

Several independent techniques were used to measure these exposure rates since each technique has its own set of limitations (for example, nonlinear energy response, portability of equipment, and extent of geographical coverage). We used results from the following techniques to determine the total exposure rates at 1 m above the ground: (1) portable, hand-held NaI scintillation detector measurements at 1,000 locations distributed over all islands, (2) LiF and CaF_2 : Dy thermoluminescent (TLD) measurements at 100 locations on selected islands, and (3) a helicopter-borne array of 40 NaI crystal spectrometers flown in a grid pattern over each island. The third technique reveals that the primary contribution to the total exposure rates was caused by ^{137}Cs and ^{60}Co activities in the soil. Soil γ spectral measurements indicate that trace amounts of other γ emitters such as ^{125}Sb , ^{155}Eu , and ^{241}Am contribute at most 3–5% of the total exposure rate¹ and, therefore, were omitted in the dose evaluation.

Because of its energy linearity, excellent thermal stability and approximate air equivalency for a typical environmental

radiation field, we selected the LiF results as the reference to which measurements obtained by the other techniques could be compared. This comparison revealed that the LiF measurements are 17% less than those of CaF_2 . This is reasonable in view of the enhanced energy response of the CaF_2 at low energies. Most importantly, however, the aerial survey and the portable NaI detector results agree, on average, within 10% with the LiF measurements. This is within the accuracy of the measurements, and the agreement is considered excellent.

As expected, the highest exposure rates were measured on the northern islands. The highest average value of $115 \mu\text{r. h}^{-1}$ was recorded on Belle. The remaining islands typically have exposure rates of $10\text{--}80 \mu\text{r. h}^{-1}$. In general, the highest levels were found at centres of the island or in proximity to ground zero sites, and were usually related in a direct way to the vegetation density in the immediate area. The southern islands are characterised by uniformly distributed levels of less than $1 \mu\text{r. h}^{-1}$ (see ref. 1 for full details of data).

External dose determination

In addition to the γ -ray exposure rates, the expected living patterns of the future inhabitants must be considered to evaluate the external dose problem. Because of the uncertainties inherent in predicting future living patterns, several cases were

Table 1 Assumed geographical living patterns

Case description	Group	% Population	Village	% time spent in respective areas				
				Beach	Interior	Lagoon	Other islands	
Ia Village on Janet, visits to other northern* islands only	Infants	22	85	5	0	0	10	
	Children	42	55	10	15	5	15	
	Men	20	50	5	15	10	20	
	Women	16	60	10	10	0	20	
Ib Village on Janet, visits to other northern* islands only	Infants	22	70	5	5	0	20	
	Children	42	50	5	15	10	20	
	Men	20	40	5	20	10	25	
	Women	16	50	5	15	5	25	
II Village on Fred, Elmer or David, visits to northern* islands only (excluding Janet)	Infants					Same as case Ib		
	Children							
	Men							
	Women							
III Village on Janet, visits to southern† islands only	Infants					Same as case Ib		
	Children							
	Men							
	Women							
IV Village on Fred, Elmer or David, visits to southern† islands only	Infants					Same as case Ib		
	Children							
	Men							
	Women							

*Northern islands include Alice, Belle, Clara, Daisy, Irene, Janet, Kate, Lucy, Mary, Nancy, Olive, Pearl, Sally, Tilda, Ursula, Vera, Wilma.
 †Southern islands include all islands from Tom to Leroy proceeding clockwise around the atoll.

chosen for analysis (Table 1). The selection was based on the most recent information available regarding present population figures, age distributions, and expected life styles¹. Furthermore, the cases were chosen in such a way as to bracket the most likely range of doses that could be received by any sizeable segment of the population (a total of 430 people). This will enable any other reasonable pattern to be inferred by proper interpolation of the results obtained for the cases shown in Table 1.

The cases are based on the assumption that some fraction of the population may choose to reside primarily on Janet (the largest island within the northern part of the atoll), with the remainder residing on Fred, Elmer, or David in the southern group of islands. Each case considered allows for visits to other islands. Case Ib differs from case Ia in that more time is allotted to temporary occupation of islands other than Janet at the expense of less time being spent in the Janet village area. These cases, or combinations thereof, are considered to represent the most likely living patterns.

Even though wide variations in γ -ray exposure rates were measured throughout the northern islands, it was necessary, for the purpose of calculating the dose, to derive the most reasonable values of the current mean exposure rates for each specific geographical area considered. These values are shown in Table 2. The mean exposure rates for specific areas of Janet were obtained by examination of the ¹³⁷Cs and ⁶⁰Co isoexposure-rate contour maps provided by the aerial survey. The village area was assumed to lie along the lagoon side of the island. The mean values given for all of the northern islands were obtained by weighting the mean exposure rates for each individual island with the area of each island. Since the minor contamination of the southern islands is relatively uniform, the mean ¹³⁷Cs and ⁶⁰Co exposure rates were chosen by inspection of the individual aerial-survey contour maps. The cosmic-ray contribution was estimated to be 3.3 $\mu\text{r. h}^{-1}$ at this latitude (11°N) and the naturally occurring radionuclides in the soil and seawater were expected to contribute an additional 0.2 $\mu\text{r. h}^{-1}$.

Integral 5, 10, 30 and 70-yr γ -ray doses for each age group were calculated for each case or living pattern described in Table 1. The results were then combined with the present population distribution, which is also shown in Table 1. Corrections were made for radioactive decay, but not for

possible weathering and subsequent deeper penetration of the radionuclides in the soil. The results of these calculations are given in Table 3 and are labelled 'unmodified.' Additional calculations were made to ascertain the effect of reasonable attempts to reduce the exposure rates on the atoll.

The first modification, labelled 'village gravelled' in Table 3, reflects the effect of covering the village areas with about 5 cm of uncontaminated coral gravel—a common practice throughout Micronesia. This action can be expected to reduce the γ -exposure rates in the village area by approximately a factor of 2. The second and third modifications are based on the assumption that clearing the islands for agricultural use and housing will result in some mixing of the topsoil. It seems that it would be practical during this period to also plough many of the more contaminated islands to a depth of 30 cm. Assuming that ploughing results in mixing rather than burying the topsoil, an average reduction in exposure rates of about a factor of 3 may be obtained. This is based on the present 3–5-cm relaxation lengths (the depth at which the activity is e^{-1} , or 37%, of the surface activity) for activity depth distribution in the uppermost soil layers of the more contaminated areas. This value, however, is highly variable from site to site. In Table 3, modification 2 indicates the effect of ploughing only Janet, whereas modification 3 reflects the additional effect of ploughing all the northern islands. Deeper ploughing

Table 2 Estimated mean exposure rates ($\mu\text{r. h}^{-1}$) used for dose calculations

Major geographical area	Source	Village	Interior	Beach
Janet	¹³⁷ Cs	9.0	33	1.0
	⁶⁰ Co	5.0	14	0.5
Fred, Elmer or David	¹³⁷ Cs	0.2	0.2	0.2
	⁶⁰ Co	0.1	0.1	0.1
Area-weighted mean exposure rates				
Northern islands (Alice–Wilma but excluding Janet)	¹³⁷ Cs	14		
	⁶⁰ Co	21		
Southern islands (Tom–Leroy)	¹³⁷ Cs	0.2		
	⁶⁰ Co	0.1		
All areas (including lagoon)	Cosmic and natural	3.5		

Table 3 Estimated integral external free-air γ doses (rad)

	Time interval (yr)			
	5	10	30	70
Ia/Unmodified	0.76	1.37	3.12	5.33
1. Village gravelled	(0.62)	(1.12)	(2.58)	(4.51)
2. + Janet ploughed	(0.41)	(0.75)	(1.77)	(3.27)
3. + Northern islands ploughed	(0.30)	(0.56)	(1.40)	(2.76)
Ib/Unmodified	0.83	1.49	3.35	5.65
1. Village gravelled	(0.71)	(1.28)	(2.89)	(4.96)
2. + Janet ploughed	(0.49)	(0.87)	(2.01)	(4.96)
3. + Northern islands ploughed	(0.33)	(0.61)	(1.50)	(2.90)
II/Unmodified	0.38	0.68	1.59	2.97
3. Northern islands ploughed	(0.22)	(0.41)	(1.08)	(2.26)
III/Unmodified	0.60	1.10	2.60	4.60
1. Village gravelled	(0.48)	(0.88)	(2.14)	(3.90)
2. + Janet ploughed	(0.25)	(0.48)	(1.26)	(2.56)
IV/Unmodified	0.14	0.28	0.83	1.92
Mean population dose (average of cases Ib-IV)				
Unmodified	0.49	0.89	2.09	3.79
1. Village gravelled	(0.43)	(0.78)	(1.86)	(3.44)
2. + Janet ploughed	(0.32)	(0.58)	(1.42)	(2.77)
3. + all northern islands ploughed	(0.24)	(0.45)	(1.17)	(2.41)
Sea level USA (80 mrad yr ⁻¹) typical	0.40	0.80	2.40	5.60

or turning over the soil rather than mixing it would, of course, result in even greater reductions in exposure rate. For example, mixing to a depth of 60 cm would reduce the exposure rates by an additional factor of 2, whereas covering the sources with approximately 30 cm of uncontaminated soil would essentially reduce the exposure rates to negligible values similar to those observed on the southern islands. Removing the top 15 cm of soil, which often contains about two-thirds of the activity, would result in a threefold reduction in the exposure rates. The advantages of ploughing or removing the topsoil should, however, be considered on a case-by-case

basis because of the highly variable distributions of activity with depth.

From Table 3 it can be seen that extensive modifications may not be required to reduce the dose levels to values comparable to typical US values². Keeping in mind that the selected cases represent approximations to the most likely living patterns, one observes that even for cases Ia and Ib, the unmodified 70-yr integral doses are comparable to the US values, although cases II and IV lead to considerably lower doses. The mean integrated doses shown in Table 3 were derived by averaging those for cases Ib, II, III and IV. This implies that half the returning population live on Janet and the other half live on Fred, Elmer or David and that trips to the northern or southern islands are equally likely for both groups. The unmodified mean population doses are all quite comparable with US values. At most, implementation of modifications 1 and 2 should be enough to assure mean population exposures well below the US levels.

Because of the low amount of natural radioactivity normally present in the coral atolls, the external dose levels calculated for cases I-III are still appreciably higher than corresponding levels found elsewhere in the Marshall Islands (essentially case IV). The results for cases II and IV indicate that restricting the permanent villages to 'clean' southern islands at least temporarily would result in lower exposures.

All of the doses discussed so far result from free-air γ -ray plus cosmic-ray exposures. The effect of shielding by structures or the body itself on gonadal or bone doses has been ignored. To convert from free-air dose (rads) to gonadal dose (rem), a body-shielding factor of 0.8 may be used³.

Received March 5; accepted August 4, 1975.

¹ Eniwetok Radiological Survey, US Atomic Energy Commission Report NVO 140, Vol. I-III (Nevada Operations Office, Las Vegas, Nevada, 1973).

² Beck, H. L., Lowder, W. J., Bennett, B. G., and Condon, W. J., *Further Studies of External Environmental Radiation*, USAEC, Health and Safety Laboratory Report HASL-170, Table IV (1966).

³ Report of the United Nations Scientific Committee on the Effects of Ionizing Radiation: Levels and Effects, Vol. 1, Levels, Annex A, 38 (1972).

Demonstration and origin of six tertiary base pair resonances in the NMR spectrum of *E. coli* tRNA^{Val}₁

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The 360-MHz NMR spectrum of E. coli tRNA^{Val}₁ reveals 26 resonances, six of which are derived from tertiary base pairs. The origin of these tertiary resonances and their approximate positions in the spectrum are discussed in relation to the crystal structure of tRNA.

Each Watson-Crick base pair generates a single low field nuclear magnetic resonance (NMR) derived from the ring NH hydrogen bond¹. Kearns *et al.*^{2,3} showed that the ring NH hydrogen bonds from base pairs in tRNA in H₂O solutions, although solvent-exchangeable, possessed

adequately long helix lifetimes to generate discrete resonances with chemical shifts in the -11 p.p.m. to -15 p.p.m. region. High resolution NMR studies of several class I tRNA species (20±1 cloverleaf base pairs) have been carried out; the spectra were interpreted to contain 20±1 low field resonances from secondary base pairs, that is it was claimed or assumed that no extra resonances from tertiary base pairs were present²⁻¹⁴.

We recently showed that, of all the class I tRNA species so far studied, *Escherichia coli* tRNA^{Val}₁ exhibited the best-resolved low field NMR spectrum; integration of the 270-MHz spectrum of this tRNA on the assumption that several resolved peaks contained a single proton, indicated

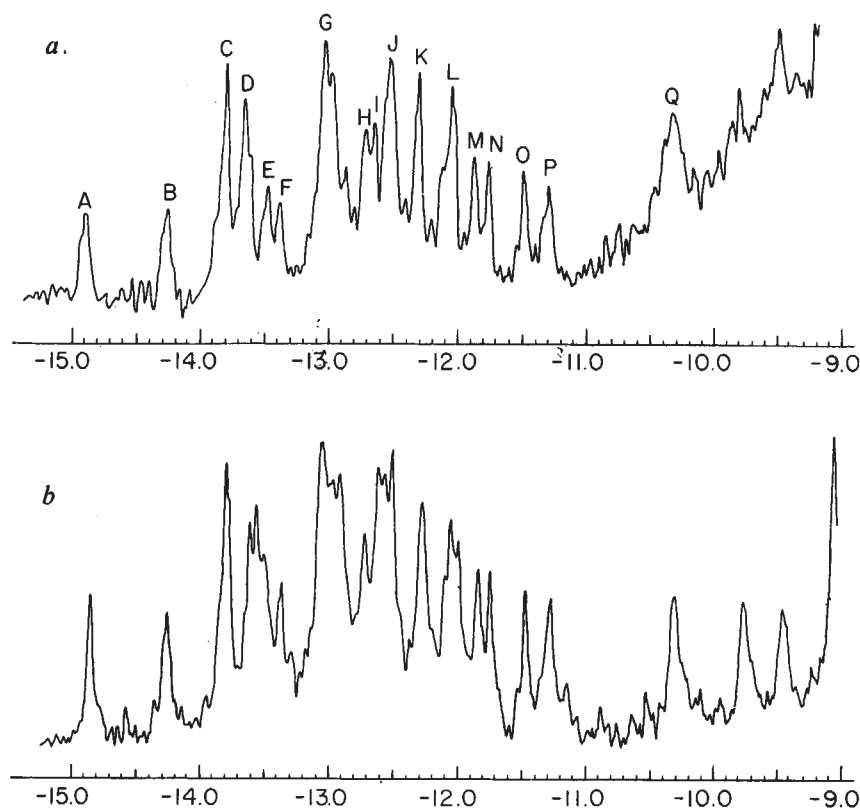


Fig. 1 360-MHz spectrum of *E. coli* tRNA^{Val} in the presence (a) and absence (b) of magnesium. The solvent for the upper spectrum was 10 mM Na cacodylate, 15 mM MgCl₂, 0.1 M NaCl, 1 mM EDTA, pH 7.0. The solvent for the lower spectrum was 10 mM Na cacodylate, 10 mM EDTA, pH 7.0. In both samples the tRNA concentration was approximately 25 mg ml⁻¹ (about 1 mM) and the spectra were signal averaged for several hours to improve signal-to-noise ratio.

that, contrary to previous interpretations, the spectrum contained several extra resonances derived from tertiary base pairs¹⁵. We have now reinvestigated *E. coli* tRNA^{Val} at 360 MHz; the improved resolution reveals six tertiary base pairs in the solution structure of this tRNA. The demonstration of 26 ± 1 base pairs is independently corroborated by calibrating the intensity of the low field spectrum with respect to the intensity of the methyl resonances of the three methylated bases in the molecule.

Internal calibration of 360-MHz spectrum

E. coli tRNA^{Val} was purified to 98% homogeneity by standard chromatographic procedures. Figure 1 shows the 360-MHz spectrum at 35 °C; in the presence of Mg²⁺ (Mg²⁺-tRNA ratio of 15) and in the absence of magnesium. In the magnesium-containing spectrum there are 16 resolved peaks between -11 p.p.m. and -15 p.p.m. In our previous spectra at 270 MHz we were able to integrate

with respect to the single proton peaks at -14.9 p.p.m. and -14.3 p.p.m. and establish that the total intensity corresponded to 26 ± 3 protons. Now, with the superior resolution at 360 MHz, we can integrate most peaks individually. Peaks A, B, E, F, M, N, O, P have the same intensity which we assume to correspond to one proton. Based on this assumption peaks C, D, K and peak H+I correspond (with an error of less than 10%) to two protons. Peak J contains three protons and peak G contains five protons; peak L corresponds to an intensity significantly greater than two protons. The experimental peak intensities and their nearest integral values are listed in Table 1. Thus integration of resolved individual peaks enables us to determine the overall intensity with much greater accuracy and leads to a value of 26 ± 1 protons. Since there are only 20 secondary base pairs in this tRNA (Fig. 3) this establishes that there are at least six tertiary base pairs involving ring N · · · H bonds in the solution structure.

Table 1 Integrated intensities of individual peaks in the 360 MHz spectrum of *E. coli* tRNA^{Val}

Peak	Position (p.p.m.)	Intensity	Integral value
A	-14.9	0.8	1
B	-14.3	1.0	1
C	-13.8	2.0	2
D	-13.7	2.1	2
E, F	-13.5, -13.4	1.9	2
G	-13.0	5.0	5
H, I	-12.7, -12.6	2.1	2
J	-12.5	3.0	3
K	-12.3	1.9	2
L	-12.1	2.4	2-3
M	-11.9	1.0	1
N	-11.8	0.9	1
O	-11.5	0.9	1
P	-11.3	0.9	1
Total			26 ± 1

Independent calibration based on methyl resonance intensity

The validity of the assumption that the resolved small peaks in Fig. 1 contain one proton can be justified on the grounds that, if they contained two protons the spectrum would indicate 54 base pairs which is impossible with only 76 nucleotides. Since the conclusion that six resonances from tertiary base pairs were present in the spectrum is, however, contrary to all previous interpretations of class I tRNA spectra, we felt it was important to calibrate independently the intensity of a single proton.

A sample of 11 mg of *E. coli* tRNA^{Val} was dissolved in 2.1 ml of water and two samples of 1.00 ml were lyophilised in separate small tubes. The first sample was dissolved in 0.20 ml of D₂O and the high field spectrum in the methyl region was taken; the second sample was dissolved in 0.20 ml of the normal H₂O buffer and the low field spectrum taken as usual. Both spectra were accumulated

for the same number of sweeps at the same r.f. power. Figure 2 shows the two spectra. There are three resolved peaks of equal intensity at -2.8 p.p.m., -2.6 p.p.m. and -1.1 p.p.m. in the region expected for methyl resonances. Figure 3 shows the cloverleaf structure of *E. coli* tRNA_{1^{Val}} which contains three methylated bases, namely m⁶A37, m⁷G46 and rT54. Thus the three methyl peaks in the high field spectrum define the intensity of three protons at the same spectrometer settings used in the low field spectrum. Peaks A, B, M, N, O, P all have an intensity between 30% and 35% of the methyl intensity, thus establishing unequivocally that they are in fact single protons. Furthermore, the overall intensity of the low field spectrum is 8.5 times the methyl resonance intensity, again corroborating the presence of 26 ± 1 base pairs.

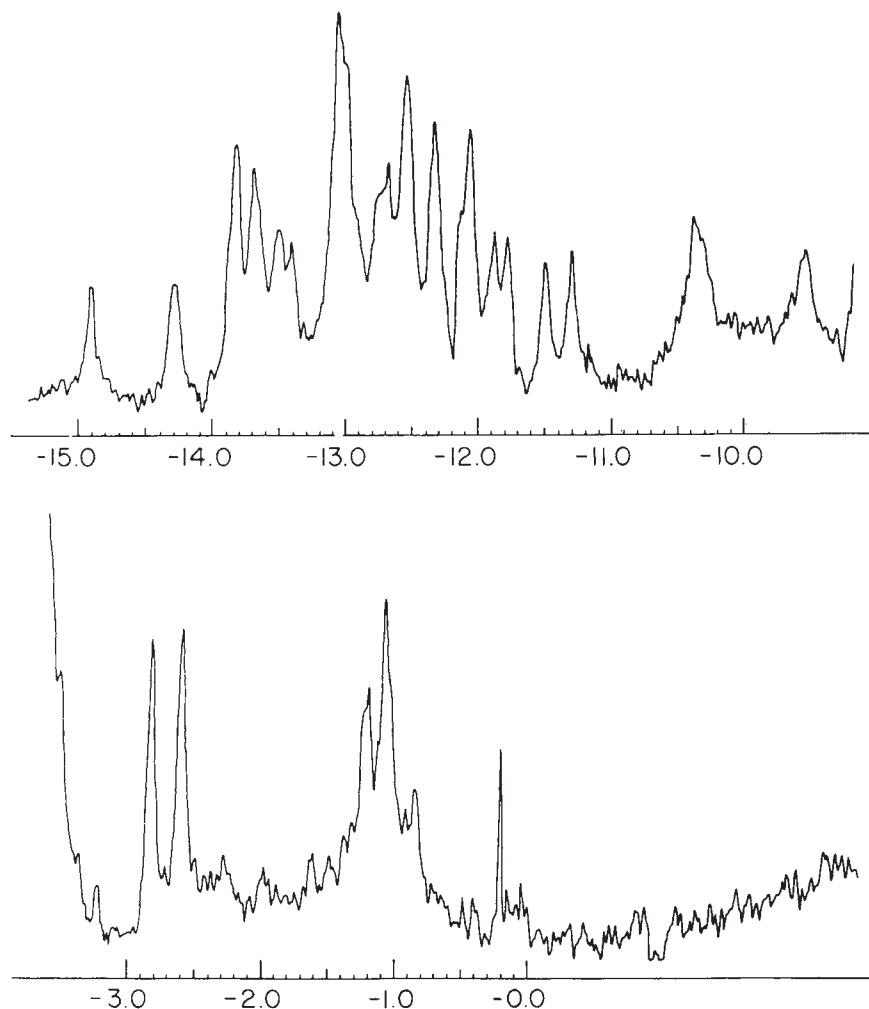
Previous assignments of methyl resonances in tRNA by Kan *et al.*¹⁶ enable us to assign the peak at -1.1 p.p.m. to rT54. The peaks at -2.8 p.p.m. and -2.6 p.p.m. must be m⁷G46 and m⁶A37 and, based on the data of Kan *et al.*¹⁶, the -2.8 p.p.m. peak can be tentatively assigned to m⁷G46. The peak at -1.2 p.p.m. has an intensity of approximately 2 protons and is probably the methylene protons of the oxyacetic side chain of residue V34 in the wobble position of the anticodon triplet. The peak at -0.2 p.p.m. is a solvent contaminant, but there is still some rather diffuse intensity between -0.7 p.p.m. and -2.5 p.p.m. which may be derived from hU17.

Base pairs contributing tertiary resonances

The crystal structure of yeast tRNA^{Phe} has been determined at 3 Å resolution^{17,18}. The fact that almost all

positions involved in tertiary bonding in yeast tRNA^{Phe} are occupied by the same nucleotide in *E. coli* tRNA_{1^{Val}}, together with more detailed arguments on the general structure of class I tRNAs^{19,20}, prompted us to consider the origin of the tertiary base pair resonances on the basis of the crystallographic data. The five known tertiary interactions involving ring NH hydrogen bonds are shown in Fig. 4. Four of these involve ring NH...ring N bonds and one (G15-C48) involves a ring NH which bonds to an exocyclic carbonyl oxygen; hydrogen bonding to carbonyl oxygens is less deshielding and would be expected to generate a ring NH resonance towards the high field end of the -11 to -15 p.p.m. region¹. There are nine tertiary interactions seen in the crystal structure^{17,18}; however, three of them (A9 to AU12; A21 to A14; G26 to A44 or A26 to G44 in tRNA_{1^{Val}}), involve exocyclic amino hydrogen bonds instead of ring NH bonds and thus would not be expected to contribute to the -11 to -15 p.p.m. region of the spectrum¹. The last tertiary pair is G18-ψ55. The precise bonding in this interaction has not been determined unambiguously; however, recent data (A. Rich, personal communication) indicate that it involves a ring NH proton which probably bonds to a ribose oxygen. Such bonding would lead to a tertiary resonance which would also be expected to be towards the high field end of the -11 to -15 p.p.m. region of the spectrum. The crystallographic data showing five definite and one probable tertiary interaction involving ring NH bonds, and the data presented here showing six tertiary ring NH resonances, are in excellent agreement. Our data are completely consistent with the crystal structure being the actual structure in solution.

Fig. 2 Low field hydrogen bond and high methyl spectra of *E. coli* tRNA_{1^{Val}} samples at identical concentrations. Duplicate samples (26.2 mg ml^{-1}) were prepared as described in the text in D₂O containing 15 mM MgCl₂, 0.1 M NaCl (lower spectrum) and in 10 mM cacodylate, 15 mM MgCl₂, 0.1 M NaCl, 1 mM EDTA, pH 7.0, H₂O buffer (upper spectrum). Both spectra were accumulated at the same r.f. power for 1,500 sweeps of 16 s each at a temperature of 38 °C.



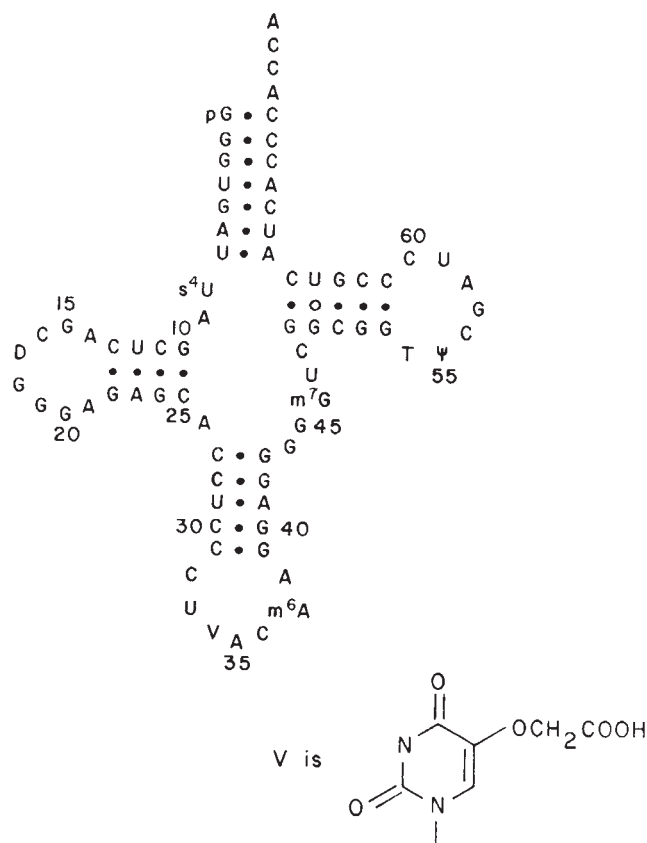


Fig. 3 The cloverleaf structure of *E. coli* tRNA^{Val} as reported by Yaniv and Barrell²³ and by Kimura *et al.*²⁴.

Expected chemical shifts for tertiary resonances

The environment of the various tertiary base pairs in the crystal structure, together with the approximate ring current shift contributions from neighbouring bases determined previously⁵, can now be used to estimate the approximate position of these tertiary resonances. The s⁴U8–A14 tertiary interaction has been assigned to peak A at –14.9 p.p.m. by chemical modification with cyanogen bromide¹⁵; additional modifications of this unique sulphur in *E. coli* tRNA^{Val} and *E. coli* tRNA^{Arg} by Wong *et al.*²¹ agree with this assignment. The reason for the extreme low field position of this tertiary resonance is the inherently

greater deshielding of the ring NH of s⁴U compared with U.

The T54–A58 interaction involves a ring NH...ring N bond. It is stacked with G18–ψ55 on one side and with G53–C61 on the other side; hence this resonance will be only moderately upfield shifted by neighbouring ring current effects. Since AT pairs have similar resonance positions to AU pairs²², we would expect the T54–A58 resonance to be somewhere in the –13.5 to –14 p.p.m. region, that is peaks C, D, E or F (peak B is in the position expected for the secondary AU6 pair).

The G19–C56 interaction is a standard Watson–Crick pair with a ring NH...ring N bond. This tertiary pair is interesting in that it constitutes one extremity of the molecule with no residue on one side and G57 stacked on the other side. Thus, this resonance would be only moderately shifted from –13.6 p.p.m. to somewhere in the –12.7 to –13.3 p.p.m. range, that is peak G, H or I.

The m⁷G46 to G22 tertiary interaction is a ring NH bond from m⁷G46 to the ring N of G22. Its environment involves stacking with A21, A14, A9 and A23¹⁹. Since adenine is the most potent ring current shift base⁵, this resonance would suffer an extremely large upfield shift—perhaps 2 p.p.m. or more. Thus the 46–22 tertiary resonance would be expected in the –11 to –12 p.p.m. region (peak M, N, O or P).

The 15–48 and 18–55 tertiary interactions involve ring NH protons which do not bond to ring nitrogens but instead bond to oxygen atoms. Such bonding has been shown to be less deshielding⁷ and in addition these bonds would suffer an average upfield ring current shift from their stacking neighbours¹⁹. Thus the 15–48 and 18–55 resonances would be expected at the high field end of the spectrum between –11 and –12 p.p.m., that is peak M, N, O or P.

To summarise, the 8–14 interaction is at –14.9 p.p.m. and the 54–58 and 19–56 interactions are probably around –13.7 p.p.m. and around –13.0 p.p.m. respectively. The crystallographic data also lead us to predict that the three remaining tertiary resonances—15–48, 18–55 and 46–22—will all be in the –11 to –12 p.p.m. region; the spectrum reveals four protons in this region and the secondary pair G5–C68 is predicted to be at –11.6 p.p.m.

Tertiary structure in absence of magnesium

The low spectrum in Fig. 1 is that of a sample dialysed for 48 h against three changes (61 each) of double distilled water, lyophilised, and then dissolved in 10 mM sodium cacodylate, 10 mM sodium-EDTA, pH 7.0, at a concentration of 1 mM tRNA. The sodium ion concentration in the

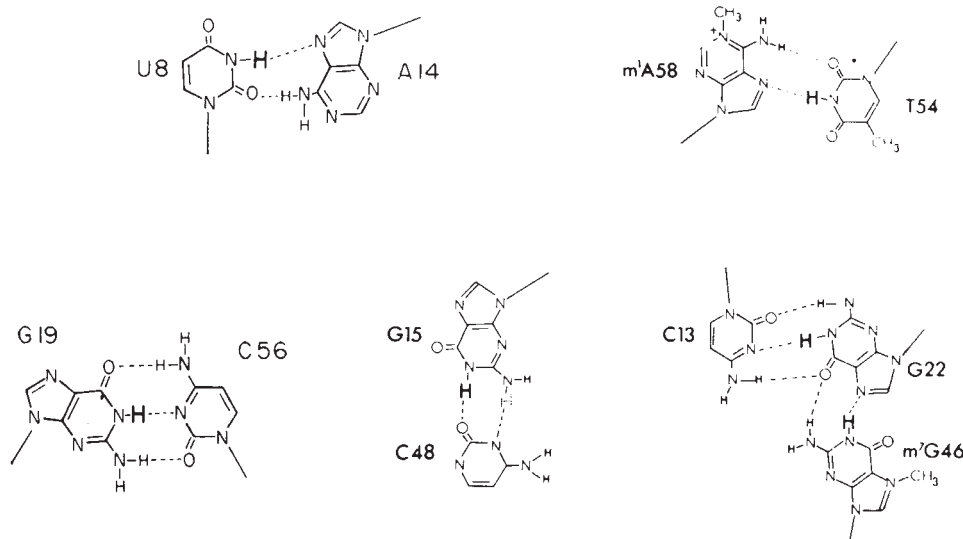


Fig. 4 Tertiary hydrogen bonding interactions seen in the crystal structure of yeast tRNA^{Phe} determined by Kim *et al.*^{17,19} and by Robertus *et al.*¹⁸ and Klug *et al.*²⁰. Only interactions involving ring NH hydrogen bonds are shown and these are denoted in bold face.

added solvent is 40 ± 5 mM. The lyophilised tRNA is the sodium salt and, at 1 mM, would generate another 75 ± 5 mM sodium ion. This excess EDTA-no magnesium solvent was chosen in the hope of destabilising tRNA tertiary structure. The 35 °C (and also the 45 °C) spectrum revealed, however, that all 26 resonances are still present. There are only two resonances which shift slightly in the non-magnesium structure; peak E (-13.5 p.p.m.) shifts downfield slightly into peak D, and peak I (-12.7 p.p.m.) shifts upfield slightly into peak J. Although it is difficult to interpret unambiguously such a subtle change, our current hypothesis is that the stacking of the acceptor helix on the rT helix (base pair 7 on base pair 49) is changed from the imperfect 17° stack¹⁷ to a more colinear stack. Concomitant with this change is a noticeable sharpening of the resonance at -10.4 p.p.m. We have assigned this resonance to the secondary G50-U64 pair¹⁵ which is presumably more protected from solvent exchange in the new structure.

The major conclusion to be drawn from these two spectra, however, is that all the tertiary interactions are formed even in the absence of magnesium; in fact the lack of spectral changes in all the other resonances suggests that the non-magnesium structure is remarkably similar to the magnesium-stabilised structure. Stabilisation of the structure by traces of residual bound magnesium can be discounted since heating the 1 mM tRNA sample in 10 mM EDTA to 65 °C for 1 h followed by subsequent cooling to 35 °C, resulted in a spectrum identical to the lower spectrum in Fig. 1.

Relation to earlier tRNA NMR data

We have now shown that *E. coli* tRNA₁^{Val}, with 20 secondary base pairs, contains six tertiary base pair resonances in its low field NMR spectrum; do the other class I tRNAs studied previously really have no tertiary resonances as claimed, or have their spectra been misinterpreted? The spectrum of yeast tRNA^{Phe} has been studied in greatest detail and has assumed the role of a 'reference spectrum'^{2-6,8-12}. The integration of low field spectra has been complicated by the lack of suitable internal standards with such large chemical shifts. Using Met-cyanomyoglobin as an external standard Wong *et al.*⁴, after normalising the two spectra to the same molar concentrations and the same number of accumulated sweeps, arrived at an intensity of 18.6 protons in the total spectrum. A composite peak at -13.7 p.p.m. was found to contain slightly more than three protons; this peak was normalised to 3.00 protons and subsequently became an 'internal stan-

dard'. A more recent revision by Kearns *et al.*¹⁰ reported a value of 19.7 protons for the total intensity of the yeast tRNA^{Phe} spectrum, but again made the assumption that the -13.7 p.p.m. peak contained three protons. We have re-examined the low field spectrum of yeast tRNA^{Phe} at 360 MHz (manuscript in preparation). At this resolution the peak at -13.7 p.p.m. resolves into three peaks of intensity 1:2:1, that is it contains four protons. Since this peak has previously been assumed to contain three protons and was used as an internal standard, it follows that the true intensity should be 1.33 times higher than reported. We note that 1.33 times 19.7 is in fact very close to 26. Independent integration of the yeast tRNA^{Phe} 360-MHz spectrum, which resolves into 14 peaks instead of the nine peaks in the 300-MHz spectrum, leads to a value of 26 ± 1 protons. Thus, we believe that all class I tRNAs probably contain approximately six tertiary resonances in their low field spectra, and previous NMR analyses have been erroneously interpreted.

We thank Susan Ribeiro and Lillian McCollum for technical assistance. This work was supported by grants from the US Public Health Service and the US National Science Foundation to B.R.R.. The use of the Dutch NMR facility at the University of Groningen and the support of the Netherlands ZWO is acknowledged.

Received June 9; accepted August 6, 1975.

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letters to nature

X-ray outburst from the direction of the galactic centre

WHEN the Uhuru satellite examined the X-ray emission from the region of the galactic centre in 1971 an extended region of emission centred on the radio source Sgr A and about 2° in extent was observed¹. We report here details of a transient point X-ray source, A1742-28, which has been observed at the centre of this region by the Ariel V rotation modulation collimator (RMC) experiment. Its rise to a peak and subsequent decline by a factor of two over 12 d were monitored. The

position was found to be $\alpha = 17^{\text{h}} 42^{\text{m}} 26.0 \pm 4.8^{\text{s}}$, $\delta = -28^{\circ} 59.8 \pm 1.2'$ (1950.0 coordinates, 90% confidence limits).

Figure 1 shows the intensity variation of the new source. Observations were made primarily in the photon energy range 3.0 to 7.5 keV although some data were obtained in the range 4.6-12 keV. Each plotted point represents observations spread over a period of one orbit (101 min). The RMC experiment is primarily designed to measure positions of point sources and has a grid pitch to separation ratio of 112, corresponding to an image response with full width at half maximum (FWHM) of

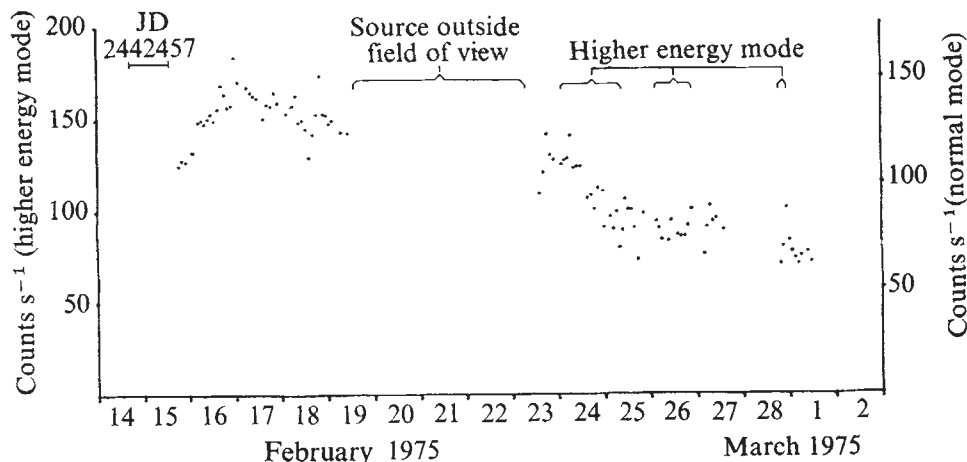


Fig. 1 The X-ray light curve of the transient A1742-28.

15' for a point source². Thus, we expect little or no response to extended sources larger than this and the intensities plotted correspond entirely to the new point source. The method of analysis occasionally leads to relatively large errors in intensity in regions as complex as that around the galactic centre where several other sources are always within the 17° FWHM field of view of the experiment. Thus, we do not consider the occasional large deviations from a smooth curve as necessarily significant.

In Fig. 1 the data points in the higher energy mode have been approximately normalised to bring them on to the same curve as the rest of the observations. This normalisation factor, together with the crude three-channel pulse height information available within each energy band have been used to deduce an approximate spectrum. Our findings are in reasonable agreement with those of J. C. Ives (personal communication) who reports a power law spectrum with a photon number index of about -3 and N_H approximately $2 \times 10^{23} \text{ cm}^{-2}$.

For comparison with counting rates quoted in the Uhuru (3U) catalog our figures should be multiplied by about 13. At the peak of about 170 counts s^{-1} the observed flux is about $4 \times 10^{-8} \text{ erg s}^{-1} \text{ cm}^{-2}$ in the energy range 3–10 keV.

Observations by another experiment on the same satellite indicate that the source was not present at comparable intensity

96 d before our observations commenced (K. Pounds, personal communication). No data are available for the period after March 1.

We have determined the coordinates of the new source using nearby X-ray sources of accurately known positions as standards. Lunar occultation positions for GX5-1 and GX3+1 are available^{3,4} and the positions of GX349+2 and GX9+1 are well established^{5,6} while 3U1700-37 has an optical counterpart. All of these sources were observed more or less regularly at the same time as the new source thus enabling checks to be made on the self-consistency of the results. From these checks we conclude that there are as yet unresolved systematic variations within our positional determinations. The size of the error box shown surrounding our best fit position in Fig. 2 results chiefly from the systematic effects, the random errors being only of the order of $10''$.

It is clear from Fig. 2 that the source lies very close to the direction of the radio source Sgr A and the associated infrared emission region. We have also observed a number of other broadly similar transient X-ray sources^{7,8}. The probability of a chance alignment of such an event with this particular direction, although difficult to estimate in retrospect, is certainly low. Given that an event occurred within the field of view of the experiment during the observations of that region, the proba-

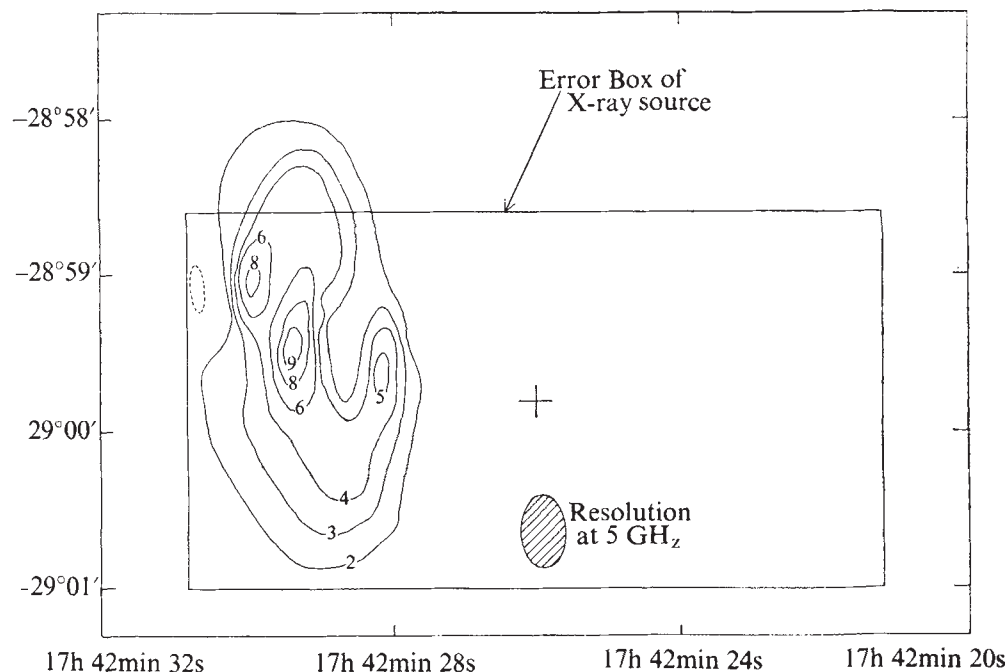


Fig. 2 The position and error box for the new source compared with a 5-GHz radio map of Sgr A west¹⁰.

bility of a chance alignment within $2'$ of Sgr A itself is $\lesssim 10^{-4}$. The probability that an event anywhere in the sky should be so aligned is of course lower than this. The probability of a similar alignment with any one of the 10 or so X-ray sources and other important features within this region is only an order of magnitude higher. It might be argued that the locally high stellar density close to Sgr A makes the occurrence of a transient similar to those observed in other regions very much higher. If the event occurred even close to Sgr A the distance must be ~ 10 kpc and this implies a peak flux of $5 \times 10^{38} \text{ erg s}^{-1}$ (3–10 kV), which is at the upper limit of the range of values usually encountered in galactic X-ray sources, perhaps suggesting again that this is a different type of phenomenon.

It is thus likely that the X-ray source is directly associated with one of the radio or infrared sources that lie within Sgr A. The fact that the rise and turnover of the light curve occurred on a time scale of the order of 2 d probably implies an emission region less than 0.002 pc in diameter. We note that long baseline interferometer studies have shown the presence of a source of less than $0.1''$ diameter (equivalent to 0.005 pc) in Sgr A west⁹, with a brightness temperature in excess of 10^8 – 10^7 K. A confirmation of this suggested association would require a change in radio or infrared emission from this region.

We thank J. C. Ives and K. A. Pounds for communicating their results before publication. We understand that a radio detection has been made by Jodrell Bank (personal communication).

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Received June 19; accepted August 15, 1975.

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Drifting subpulses and pulsar slowdown rates

MANY models^{1–3} have been proposed to explain the observed radiation from pulsars, but none has yet explained satisfactorily all the features of pulsar emission. Studies of the relationships between pulsar properties may help in the understanding of the nature and evolution of pulsars and we present here evidence for a close relationship between the direction of drifting of subpulses from a pulsar and its position in a P – $\dot{P}$ diagram—the plot of the derivative of a pulsar's period, $\dot{P}$, against period, P .

In such a diagram (Fig. 1), derived from the data of Lyne, Ritchings and Smith⁴, pulsars in the top, left hand corner of the diagram are thought to be young. They evolve downwards and towards the right hand edge of the triangular distribution of points where the pulse emission mechanism breaks down and the pulsars 'die', for reasons predicted by the various models. Interestingly, those pulsars with complex pulse profiles or

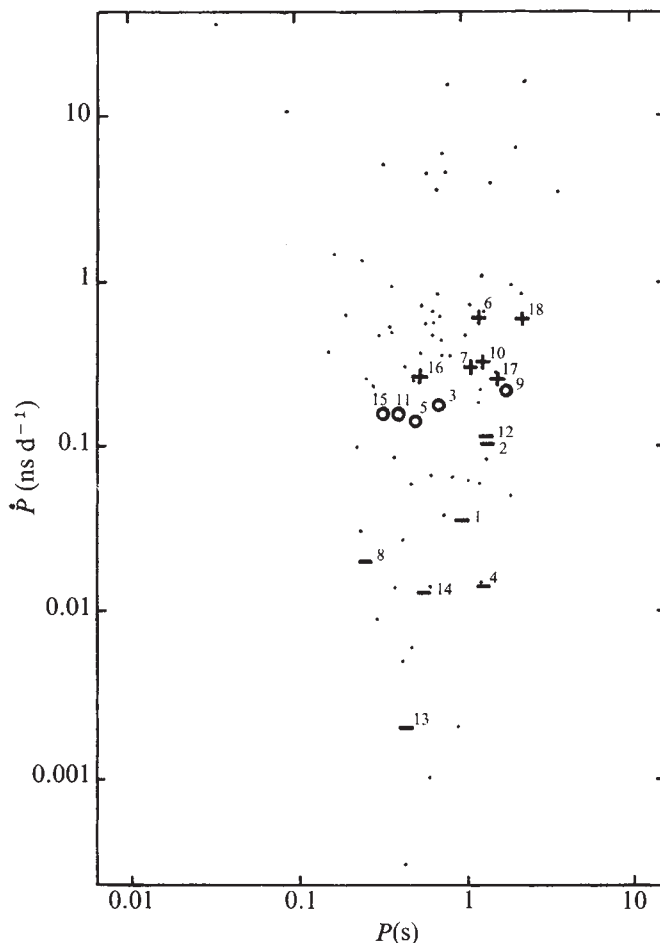


Fig. 1 A plot of the period derivative, $\dot{P}$, against the period, P , for 84 pulsars⁴. +, Subpulses drifting later, —, drifting earlier; O, drifting in both directions. Numbers correspond to those given in Table 1.

drifting subpulses, which may be a sign of pulsar 'old age'⁵, lie close to that edge.

Single pulse observations of several pulsars have been made using the Mark 1A radio telescope at Jodrell Bank, and sequences of pulses showing subpulse drift have been recorded for the pulsars PSR1112+50, PSR1642–03, PSR1944+17 and PSR2319+60. The subpulses received from PSR2319+60 drift later, that is, they drift from the leading edge of the integrated pulse profile towards the trailing edge, whereas those from PSR1944+17 move in the opposite direction. PSR1112+50 and PSR1642–03 display sequences in which the subpulses drift in either direction. These 4 pulsars, together with 14 others whose subpulse drift has been discussed in the literature are listed in Table 1. Subpulse drift from PSR1237+25 has been reported by Backer^{6,13}, but in view of the comments of Taylor *et al.*⁷, it has not been included in Table 1. Each pulsar in Table 1 is also indicated in Fig. 1, and the direction of the subpulse drift is shown.

From Fig. 1 there seems to be a trend indicating that subpulses from pulsars of high $\dot{P}$ drift later and that those from low $\dot{P}$ pulsars drift earlier. The change in drift direction occurs at $\dot{P} \sim 0.2 \text{ ns d}^{-1}$. The pulsars showing drifting subpulses have been selected by observers principally on the basis of flux densities, since a good signal-to-noise ratio is required for the detection of any drift. It is not evident, however, how that or any other selection effect could give rise to the trend, although we cannot rule out completely the possibility that it is produced by the insufficient sampling of pulsars which have a random distribution of drift directions.

It is widely believed that subpulses are associated with

discrete sources of emission and that subpulse drift is caused by the sources moving asynchronously with the pulsar rotation rate, although it is not generally clear what determines their motion. The direction of motion of the sources of emission has been defined, however, only in the model of Ruderman and Sutherland³ and then the observed direction of drift will depend on the observer's line of sight relative to the pulsar magnetic pole and the rotation axis. It would be remarkable if a chance set of orientations has produced the effect shown in Fig. 1, and subsequent studies of the subpulse drift in more pulsars may exclude this possibility.

It seems that some form of dragging on the particle bunches

lead to a better understanding of the physics of pulsars.

R.T.R. acknowledges financial support from the Science Research Council.

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Received July 24; accepted August 12, 1975.

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Table 1 Pulsars with drifting subpulses

Pulsar	Pulsar number in Fig. 1	Drift direction	Ref.
0031-07	1	—	7,9
0301+19	2	—	9,11
0329+54	3	0	12
0809+74	4	—	7,9
0823+26	5	0	8
0834+06	6	+	7
0943+40	7	+	9,10
0950+08	8	—	8
1112+50	9	0	This paper
1133+16	10	+	7
1642-03	11	0	This paper, 6
1919+21	12	—	7,8
1944+17	13	—	This paper, 9
2016+28	14	—	7
2020+28	15	0	9,11
2021+51	16	+	7
2303+30	17	+	8,10
2319+60	18	+	This paper

+, Subpulses drifting later; —, subpulses drifting earlier; 0, subpulses which drift in both directions.

responsible for the subpulse radiation may be associated with the pulsar braking. This may not be significant for those pulsars with low values of $\dot{P}$ (weak braking) and subpulses would drift earlier, but for high values of $\dot{P}$ the dragging could be large and the particles may be 'forced' to move with different speeds, possibly in the opposite direction, thus producing the variation of subpulse drift direction with $\dot{P}$ seen in Fig. 1. The exact nature of the association between the braking and dragging is not clear, but may be concerned with the magnetic dipole moment of the pulsar.

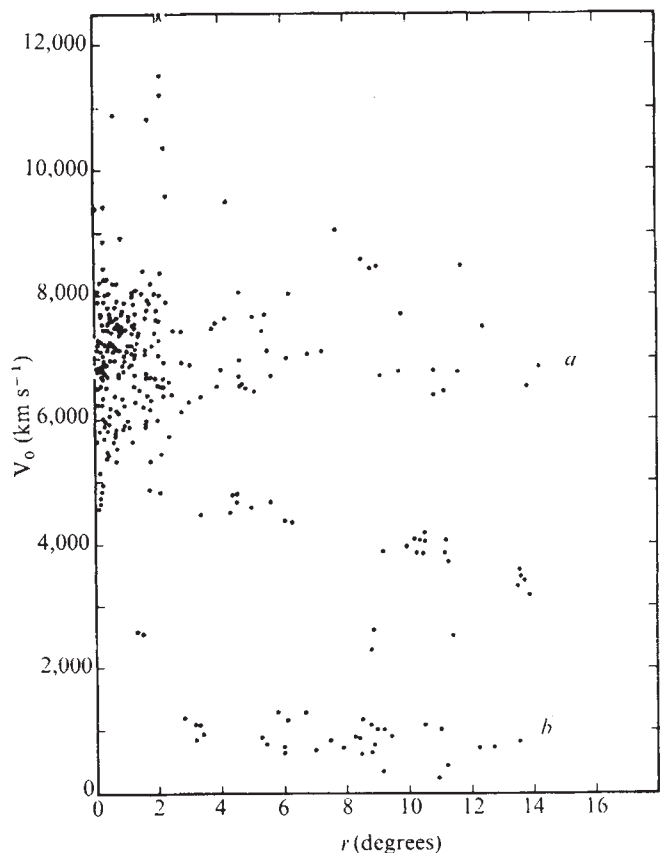
The dipole moment, M , is generally thought to be related to the rate of slowing down of the pulsar, according to $\dot{P} \propto M^2 P^{2-n}$, where n is the pulsar braking index which has experimental and theoretical values in the range 2.33-3 (see refs 1-4). In a plot such as Fig. 1, the evolutionary paths of pulsars would then be straight lines with slopes of between -0.33 and -1 and the wide range of $\dot{P}$ would result from a spread in initial values of the dipole moment. If that is the case it would seem that subpulses drift later in pulsars with large dipole moments and in the opposite direction for pulsars with small dipole moments. It has been suggested that the evolution of pulsars involves a decrease in the effective dipole moment. That would happen if the pulsar magnetic field decayed¹⁴ or realigned¹⁵, or if its configuration in the magnetosphere became more complicated¹⁶ as the pulsar grew older. Then, the pulsar evolutionary lines would be straight initially but would later deviate to lower values of $\dot{P}$, eventually becoming almost vertical. Again, it seems that the subpulses drift later while the magnetic dipole moment is strong, but change their direction of drift when it becomes weaker in the later stages of the lifetime of the pulsar.

The relationship suggested here is based on the observation of a limited number of pulsars. Further observations are clearly required to test this apparent relationship, which may

Size of the Coma cluster

STUDIES of counts of galaxies in large regions centred on the Coma cluster led Zwicky to conclude that the radius of the Coma cluster is at least 6° (13 Mpc for $H = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$). Outside this radius, the contribution to the counts from fore-

Fig. 1 Line of sight velocity relative to the Local Group against radial distance from the centre of the Coma cluster. *a*, Coma cluster; *b*, Local Supercluster.



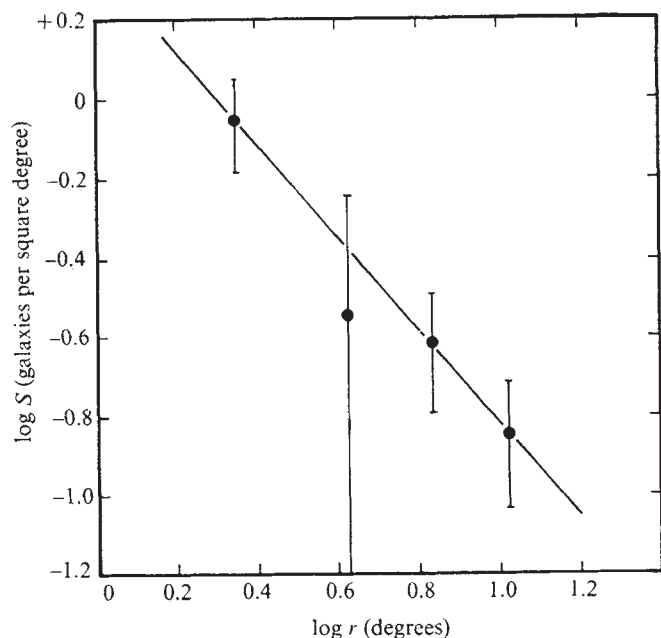


Fig. 2 Logarithm of the average number of galaxies in the Coma cluster with $m_p \leq 15.0$ mag per square degree against logarithm of the average radius of an annular ring. Error bars indicate the mean errors of sampling. The straight line has a slope of -1.18 .

ground and background galaxies overwhelms the contribution from the cluster, rendering it invisible. The technology of image intensifier tubes has now advanced to such an extent that it is feasible to obtain line of sight (radial) velocities of large numbers of galaxies in fields of a cluster. These velocities can be used to distinguish cluster members from foreground and background galaxies². Such studies have confirmed that the Coma cluster extends to at least 5° (ref. 3).

The reported study here, which completes work begun in 1972 (ref. 4), concentrates on a region $5\text{--}13^\circ$ west of the centre of the Coma cluster called cluster number 16 in field 158 by Zwicky and Herzog⁵. Spectrograms obtained with a Carnegie image tube on the 2.1-m telescope at the Kitt Peak National Observatory have been used to complete the determination of radial velocities for 50 of the 52 galaxies in cluster 16 with apparent magnitude $m_p \leq 15.0$ mag (a homogeneous sample). Some additional velocities of bright galaxies just outside the boundary of cluster 16 were also obtained. These data are used in conjunction with velocities for galaxies within 5° of the centre of the Coma cluster⁶⁻⁸ to plot radial velocity (relative to the Local Group), V_0 , against distance from the centre of the Coma cluster, r (Fig. 1).

Cluster 16 is clearly resolved into three components: (1) part of the Local Supercluster (V_0 between 200 and $1,300 \text{ km s}^{-1}$), (2) groups with V_0 between 2,200 and $4,200 \text{ km s}^{-1}$, and (3) the Coma cluster with velocities in the range $6,300\text{--}9,100 \text{ km s}^{-1}$. Galaxies in the Coma cluster with radial distance up to 14.2° are recognised, so the Coma cluster has been detected to $r = 32 \text{ Mpc}$.

Seventeen galaxies (34%) of our homogeneous sample are cluster members. Fourteen of these can be categorised into morphological types⁹, and 7–8 (50–57%) are spirals. This fraction is consistent with the tendency for the relative number of spirals to increase with radial distance found for the inner 2.79° of the cluster⁶.

The data of our homogeneous sample have been combined with other homogeneous data⁶⁻⁸ to derive the average surface density (galaxies with $m_p \geq 15.0$ mag per square degree) against distance from the centre of the Coma cluster for the r interval $1.67\text{--}12.4^\circ$ (Fig. 2). A χ^2 fit of the data to a power law reveals that the surface density is proportional to $r^{-1.18 \pm 0.24}$, which

is consistent with the formula for an isothermal sphere found to apply in the inner regions of the cluster^{1,10-12}. If the empirical formula for surface density applies to even greater radial distances, then we expect 12% of a sample of galaxies with $m_p \leq 15.0$ mag at $r = 20^\circ$ (and 4.5% at 45°) to be members of the Coma cluster.

Our homogeneous sample, which is centred at $r = 8.5^\circ$, has a mean radial velocity of $7,639 \text{ km s}^{-1}$ and a standard deviation about this average of 751 km s^{-1} . The inner region, of the Coma cluster, radius 2.79° , has a mean radial velocity of $6,981 \text{ km s}^{-1}$ and a standard deviation of 920 km s^{-1} . The greater mean velocity of our homogeneous sample (by 2.4σ) relative to the inner region of the cluster may be a statistical fluctuation. If cluster members just outside the region of the homogeneous sample are included, then the average velocity reduces to $7,374 \text{ km s}^{-1}$.

An outer radius for the Coma cluster of at least 32 Mpc makes it one of the largest known cosmic entities. Its size is at least as great as the size of the Local Supercluster and typical groups of galaxy clusters (H.J.R., unpublished). A galaxy moving with a velocity of $900\sqrt{3} \text{ km s}^{-1}$ would take 2×10^{10} years to travel 32 Mpc. Thus gravitational mixing of galaxies between the inner and outer regions of the Coma cluster is not well advanced, and the properties of the outer regions reflect to a significant extent the initial conditions of the cluster formation period following the origin of the Universe.

The density of rich clusters of galaxies is of the order of 0.8×10^{-6} clusters Mpc^{-3} . If the Coma cluster is a fairly typical cluster, as it seems to be^{13,14}, the new findings require a mass of $M(\text{Coma}) \geq 1.3 \times 10^{16} M_\odot$ and a density of the Universe $\geq 7.1 \times 10^{-31} \text{ g cm}^{-3} = 0.12(3H_0^2/8\pi G) = 0.12\rho_c$.

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Possible origin of lunar magnetism

ANY model of the origin of lunar magnetism needs to explain two principal observations¹. One is the magnitude of the stable natural remanent magnetisation (NRM) carried by the returned lunar samples, which is far too large to have been acquired in fields comparable with those found at present in the vicinity of the Moon. The other is the presence of remanent fields at the lunar surface, which imply source regions of tens or hundreds of kilometres of homogeneous magnetisation, primarily in the highlands.

Attempts to explain these phenomena include the suggestion that the Moon had, early in its history, a regenerative dynamo like the Earth's, so that the magnetisation now observed was acquired in its field². It has also been proposed that the Moon

was exposed to an external field in which the observed magnetisation was acquired³, and Urey and Runcorn⁴ and Strangway and Sharpe⁵ have conjectured that the Moon was magnetised, more or less homogeneously, early in its history, and that the present magnetisation was acquired in this remanent field. The primitive remanent field was later thermally demagnetised as the Moon heated up internally. Finally, there are models in which local and transient fields are invoked. These models tend to merge into zero-field or 'bootstrap' models which require fields no larger than the present field associated with the solar wind. Here I present another possibility, which is a variant of the remanent field models already proposed.

I suggest that the Moon acquired a primitive magnetisation during its formation by accretion from planetesimals. Two mechanisms of magnetisation are likely to have been predominantly important. First, thermoremanent magnetisation would be acquired by material which was heated above its Curie point during impacts and subsequently cooled. Second, shock magnetisation would be acquired by material which was shocked but not heated significantly by the impacts⁶.

The nature of the fields in which the proposed magnetisation was acquired remains obscure. At an early stage of the Solar System an enhanced solar wind field is not, however, implausible. Also, because the mechanisms of thermoremanent and shock magnetisation are efficient, it is not necessary to propose as large a field as in the isothermal remanence model⁵. Finally, if these ideas are combined with those of A. E. Ringwood's recent models of the origin of the Moon (unpublished), it seems possible to invoke an early geomagnetic field as a source field for the primitive magnetisation of the Moon. In this view, the Moon accretes from a ring of planetesimals in a near-Earth orbit, so that if this process took place after the formation of the Earth's core, as seems likely, the terrestrial dynamo may already have been generating a geomagnetic field.

Whether an impact, or an external field origin is invoked, the primitive magnetisation of the Moon is likely to have been inhomogeneous. Its magnitude and direction would have been controlled by the geometries of impacts and of ambient fields. The latter would change with the proto-Moon's position in its orbit. In this respect, my suggestion differs from earlier models⁵ in that they propose a more or less homogeneously magnetised Moon. My explanation derives in part from an earlier suggestion made by Meadows⁷ to account for the magnetism of meteorites.

The chief difficulty with the remanent field approach lies in explaining any very strong NRM carried by the returned samples, which would imply inducing fields of the order of 1 oersted. It is not yet clear whether a significant fraction of the returned samples carry such NRM, so this may not be a critical difficulty. Nevertheless, if large numbers of samples do turn out to imply such strong inducing fields, the present suggestion will be seriously weakened. It will then be necessary to invoke somewhat *ad hoc* field amplification mechanisms to account for the high fields, compared with the fields of about $10^3\gamma$ which remanent field models seem capable of giving at the surface.

My suggestion follows the earlier remanent field models^{4,5} in the explanation of the loss of the primitive magnetisation by later internal heating of the Moon. The field could, however, be maintained by an early cool Moon at least long enough for the crust of the Moon to be magnetised when it was formed. This magnetisation of the crust would have been primarily a thermoremanent magnetisation (TRM), but shock magnetisation is also likely to have been involved since impacts took place while the field was present. A critical prediction is that the primitive magnetisation of the Moon was inhomogeneous on a Moon-wide scale, so that the magnetisation of the crust would also be inhomogeneous on the same scale. It certainly could, however, be homogeneous over scale lengths of hundreds of kilometres. This prediction can probably be tested by the magnetometer experiment on the Lunar Polar Orbiter.

If magnetisation by the process suggested here is important in the Moon, then it should also be so in other bodies of the

Solar System which were formed in the presence of magnetic fields. If the body remained cool, a record of the process should be preserved. If the body subsequently heated up internally, the magnetisation should have been lost and the surface record of the primitive magnetisation progressively obscured by later impacts and other effects. These may take place in very weak fields and thus demagnetise material, or in later dynamo fields, in which case new patterns of magnetisation will be generated.

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Received June 23; accepted July 29, 1975.

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Recent crustal movements along Mediterranean coastal plain of Israel

THE high density of population along the Mediterranean coast of Israel dictates caution in the siting of power stations and major engineering structures. One of the critical questions is whether the coastal plain of Israel may be assumed to be tectonically quiet and stable. Lately¹, evidence was presented of Recent (probably later than 700 BP and certainly not earlier than 3,700 BP) faulting along the coast, and the coastline was assumed to be tectonic in origin, in contrast to the previously held notions of tectonic calm and a coastline shaped by wave abrasion^{2,3}. Though unable at this stage to comment on the regional implications of this controversy, we report that repeated precise levelling suggests the present occurrence of differential crustal movements along the coastal plain and the nearby Judea mountains.

Our data, based on 251 twice-levelled benchmarks aligned in seven complete and three incomplete polygons (Fig. 1), include the computed differences, between differences in elevation between consecutive benchmarks, determined in the 1962 and 1969 circuits in levelling ($dH = \Delta H_{1962} - \Delta H_{1969}$). Monumentation and geodetic operations conformed to specifications of first order precise levelling^{4,5}, discrepancy between the forward and backward measurement not exceeding $3\sqrt{D}$ mm (D is the levelled distance in km). The dH values are not related in sign nor magnitude to the discrepancies, and in most cases are significantly larger.

Figure 1 shows the locations of the studied benchmarks and the range of the computed dH values. Tide data are lacking and thus the dH values can not be related to the mean sea level. Instead, they are presented relative to a reference benchmark, with respect to which all other benchmarks subside (zero point in Fig. 1). The numbers shown on the map indicate in mm the lower and upper limits of the 1962–69 relative subsidence for each closed polygon. Arrows show the direction of tilt, and crosses indicate even subsidence relative to the reference benchmark.

Figure 1 indicates a pronounced regional tilt to the south-east and east-south-east. The differences between the adjacent polygons vaguely suggest a possible block-structure, related to the E–W trending normal faults revealed in gravity surveys of the coastal plain^{6,7}. Rate of movement and rate of tilting increase southwards. Polygons I, II, and III show a relatively gentle movement, the northernmost one subsiding evenly and

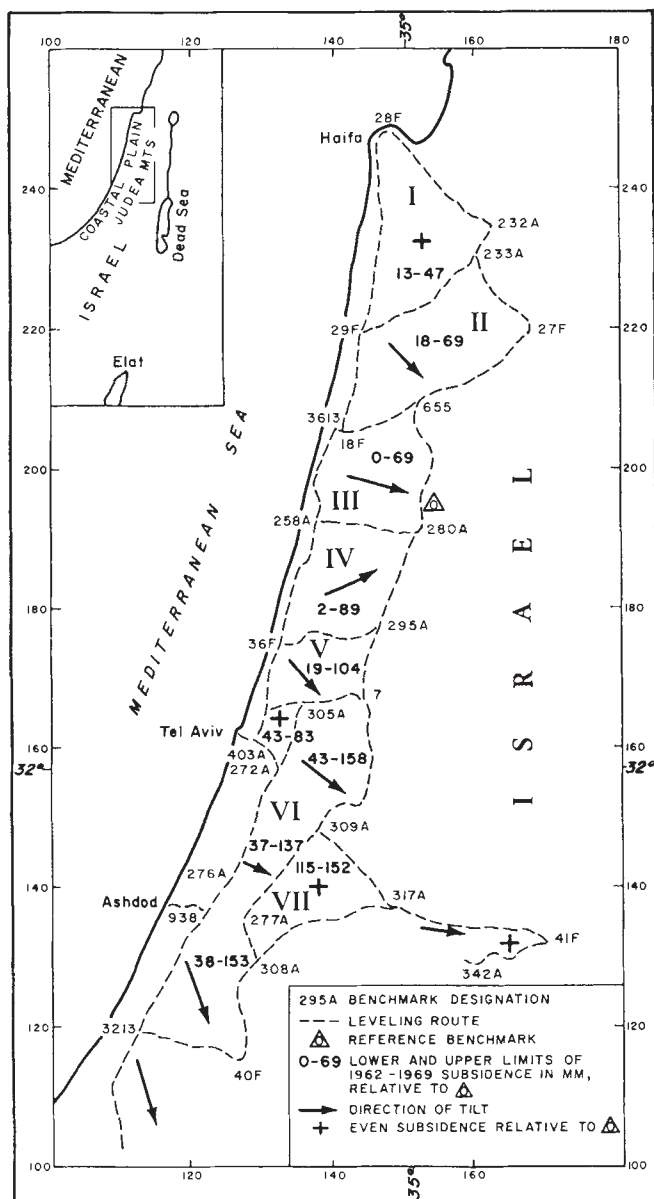


Fig. 1 Area of survey, showing variations in tilt.

the other two tilted towards ESE and SE. In polygons V and VI, tilts are stronger and the dH values reach 150 mm (an inferred movement of about 2 cm yr^{-1}). The strong easterly component of tilt suggests that the Judea mountains anticlinorium subsides relative to the coastal plain. This tendency of structural highs to sink, and of structural lows to rise, was reported also from other parts of the region⁸ and was tentatively attributed to gravity-compensation effects. It is not clear whether the southerly increase in rate of movement and tilt is tectonically controlled, or is caused by another factor such as differential loading due to a southerly increase in thickness of the sediments derived from the Nile^{9,10}.

Geological structure of the coastal plain is unexposed and structural details must be inferred from geophysical surveys and borehole correlations, thus disallowing a more detailed evaluation of the possible relationship between the dH movement of individual benchmarks and their structural setting.

The magnitude of inferred vertical crustal movements reported here is very considerable and must await verification by further levelling especially as only 25% of the benchmarks are anchored in bedrock. The time interval between the studied levellings is too short to reveal whether the dH values reflect a

consistent long term trend, or whether they are but a part of short term oscillations. But geological evidence of young tectonic activity in Israel reported in the past, and confirmed by seismological data^{11,12} indicates that the possibility of significant present day strain accumulation cannot be ignored.

We thank L. Fish and U. Shoshani (Israel Department of Surveys) for help. Assistance from Day Fund (US Academy of Sciences) and SUNY Research Foundation is acknowledged.

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Received July 23; accepted August 12, 1975.

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Influence of mantle CO_2 in the generation of carbonatites and kimberlites

THE explanation of why partial fusion of mantle peridotite sometimes yields basalts, sometimes kimberlites and sometimes carbonatites is given by the phase relationships in the system $\text{CaO-MgO-SiO}_2\text{-CO}_2$. The key is the intersection of a sub-solidus carbonation reaction with the solidus, which introduces carbonates as primary minerals alongside silicates on the surface of the liquidus.

The mineral assemblage forsterite(Fo)+orthopyroxene(Opx)+clinopyroxene(Cpx) in the system CaO-MgO-SiO_2 has been used by Kushiro¹ as a model for mantle peridotite, both dry and in the presence of H_2O , and by Eggler² in the presence of CO_2 . The P - T diagram of Fig. 1 compares the solidus curve for the mineral assemblage free from volatiles with the melting curves produced by solution of H_2O or CO_2 . At 20 kbar, the melting temperature is lowered through more than 400°C by the solution of about 20 weight % H_2O , and through 75°C by the solution of about 5 weight % CO_2 . The model mantle peridotite yields a haplobasaltic liquid that is forsterite-normative. In the presence of H_2O the liquid is enriched in SiO_2 and it is quartz-normative at 20 kbar (ref. 1). In the presence of CO_2 the liquid is depleted in SiO_2 , and somewhere between 15 and 30 kbar it becomes larnite-normative², a haplokimberlite magma.

Our new data between 25 and 35 kbar show the solidus in the presence of CO_2 sweeping down through 400°C through a pressure maximum at about 32 kbar, and terminating at invariant point Q_6 near 25 kbar and $1,200^\circ\text{C}$. This is the point where the subsolidus carbonation reaction for the mantle mineral assemblage (Fig. 2) terminates by addition of a liquid phase. Increase in pressure not only stabilises carbonate in the subsolidus mineral assemblage, but also in the liquid phase; this produces a large increase in CO_2 solubility and a dramatic change in melting temperature.

Figure 2 is an isobaric isothermal diagram showing the compositions of the minerals involved in the subsolidus carbonation reaction at mantle pressures, with dots giving the compositions of pyroxenes and carbonates coexisting across solvi³⁻⁵. On the high pressure side of the reaction, the assemblage Fo+Opx+Cpx reacts to produce Cd (solid solution between calcite and dolomite) at the expense of Cpx. This reaction was reported in an

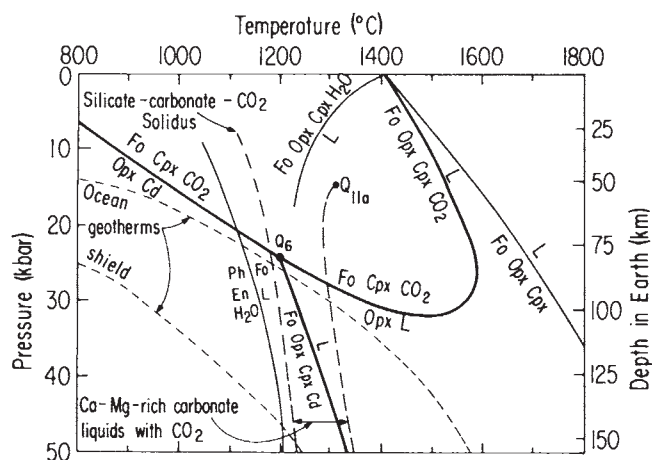
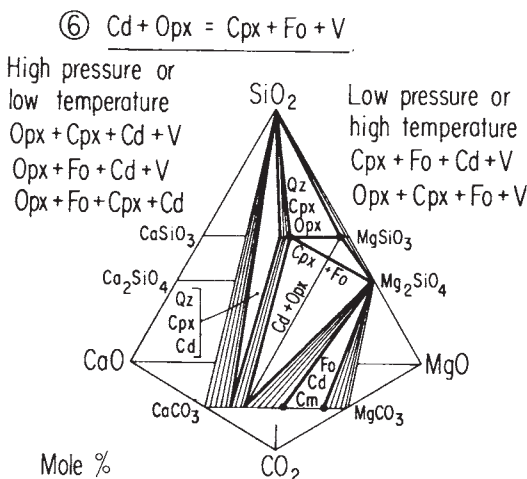


Fig. 1 The melting curve for mantle peridotite assemblage forsterite(Fo) + orthopyroxene(Opx) + clinopyroxene(Cpx), compared with reactions in the presence of H_2O , and with a small proportion of CO_2 , either as vapour phase at lower pressures, or as crystalline carbonate at higher pressures. Cd or Ccd = Solid solution between calcite and dolomite; L = liquid; Ph = phlogopite; En = enstatite. Of particular interest is the dramatic change in CO_2 -melting reactions between 25 and 35 kbar. (The numbering system, Q_6 , Q_{11a} , refers to a detailed account of the whole system, now in preparation.)

abstract by Eggler, but with a magnesian carbonate⁶. The solidus curve rising to higher pressures from Q_6 involves silicates and the carbonate. The first liquid produced is a haplo-carbonatite with about 90% by weight of carbonate.

Figure 3 shows the CO_2 -saturated liquidus surface in CaO-MgO-SiO₂-CO₂ at 28 kbar (refs 5-8 and our unpublished work). For silicates in the area SiO₂-CaSiO₃-MgSiO₃, CO_2 solubility remains low, but for compositions ranging from the pyroxene join to the carbonate join, the CO_2 content of liquids increases significantly. Note the position of the field boundary between the silicates and the carbonate solid solutions, and the liquidus minimum at point m on this surface. This minimum (and related reactions at other pressures) is represented in Fig. 1 by the dashed line solidus at temperature slightly below Q_6 . This is close to the melting curve for phlogopite coexisting with mantle minerals and H_2O (ref. 9). Figure 1 shows the range of existence for haplo-carbonatites with compositions in the general area

Fig. 2 Isobaric isothermal diagram showing mineral compositions in the univariant carbonation reaction for the mantle mineral assemblage. This is the reaction terminating at invariant point Q_6 in Fig. 1, with the addition of a liquid phase. All phase assemblages shown coexist with CO_2 (V = vapour).



between m and a in Fig. 3. The solidus reaction without CO_2 rising to higher pressures from Q_6 would be represented in Fig. 3 by a point behind the liquidus surface, somewhat further away from the CO_2 apex than liquids m and a , but with similar high contents of dissolved carbonates.

At 28 kbar (Fig. 1) the melting reaction for the assemblage $Fo + Opx + Cpx + CO_2$ is intersected twice, and the liquid compositions are represented in Fig. 3 by the higher temperature liquid b (haplokimberlite) and the lower temperature liquid a (haplo-carbonatite). With increasing pressure, points a and b approach each other, becoming coincident at about 32 kbar (Fig. 1). At greater pressures, the CO_2 -saturated liquidus fields for forsterite and clinopyroxenes are separated by the field for orthopyroxene.

The peridotite carbonation reaction in Figs 1 and 2 occurs at lower pressures than the forsterite carbonation reaction in the system $MgO-SiO_2-CO_2$ as determined by Newton and Sharp¹⁰. According to the geotherms¹¹ and the reaction in Fig. 1, we confirm their conclusion that free CO_2 cannot exist in the upper mantle except in conditions of unusually high temperature. CO_2 exists in the upper mantle as carbonate^{10,12}. The reaction is displaced to lower temperatures in the presence of diluted

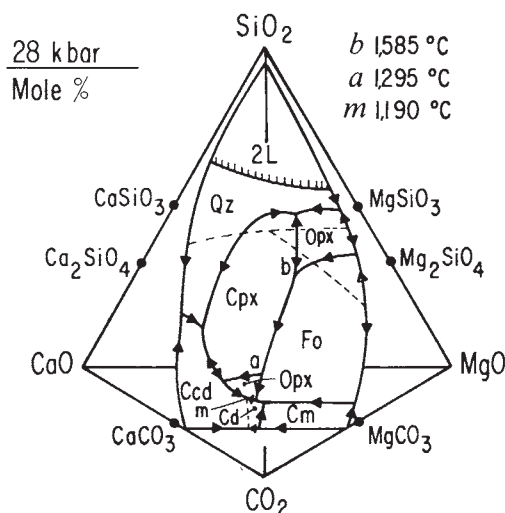


Fig. 3 CO_2 -saturated liquidus surface in CaO-MgO-SiO₂-CO₂ at 28 kbar, partly schematic. The surface gives the compositions of liquids that can coexist with free CO_2 , and shows the fields for primary crystallisation of minerals. Cm, Cd, and Ccd are carbonate solid solutions; Qz = quartz; 2L = immiscible liquids. Note the continuous crystallisation or fusion paths between haplo-basalt (liquids in area olivine-pyroxenes), haplokimberlite, liquid b , and haplo-carbonatite, liquid a .

CO_2 , however, so CO_2 could exist within a restricted depth interval in a CO_2-H_2O vapour phase, probably of limited compositional range.

The seismic low velocity zone may be caused by incipient melting due to the presence of traces of H_2O in the upper mantle^{13, 14}. Green concluded that melting was not involved, and that the low seismic velocities were caused by exsolution of CO_2 from dry silicate minerals^{15,16}. Figure 1, however, shows that mantle peridotite with CO_2 must begin to melt in the depth range 80-130 km.

In the presence of CO_2 , partial fusion of mantle peridotite at depths shallower than 80 km produces basaltic magmas, which become more undersaturated with silica at increasing depth². At depths greater than 80 km, the first magmas produced, in small quantities, are carbonatites. With progressive fusion these are converted to kimberlites and eventually, at higher temperatures, to basalts.

Any of these magmas, with fractional crystallisation at constant pressure, can yield carbonatite as residual magma at

considerably lower temperatures^{6,7}. Kimberlites or carbonatites rising from the low velocity zone must evolve CO₂ as they reach the reaction boundary at depths between about 100 and 80 km. This would certainly contribute to the explosive emplacement of kimberlites.

This work was supported by the NSF.

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Received July 2; accepted July 22, 1975.

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Palaeogeothermal gradients derived from xenoliths in kimberlite

GREAT problems exist in the determination of an ancient geothermal gradient. An attempt has been made by Boyd¹, based primarily upon the equilibration conditions of co-existing pyroxenes in garnet lherzolite xenoliths from the Thaba Putsoa, Letseng and Mothae kimberlite intrusions in northern Lesotho. He derived the equilibration temperature of the xenoliths from the amount of enstatite in solid solution in the diopside², and estimated the pressure from the amount of Al₂O₃ in the orthopyroxene³. He argued that, for a group of lherzolite xenoliths, a line joining a series of pyroxene-derived pressure/temperature points should define the geothermal gradient in the mantle below northern Lesotho during the Cretaceous when the xenoliths were entrained by the kimberlite during its ascent. He found a Cretaceous gradient similar to that in present-day shield areas between depths of 100 and 150 km, though from 150 to 200 km depth it was considerably steeper. Moreover, whereas the lherzolites outlining the shallower 'normal' part of the gradient are of granular texture, those outlining the high-temperature, steeper and deeper segment exhibit a range of deformation and recrystallisation textures (the porphyroclastic and mosaic textures in the classification scheme of Bouillier and Nicolas⁴).

Accordingly, it has been proposed^{1,5} that the perturbation of the normal subcratonic gradient was caused by stress heating at the base of the African Plate during the breakup of Gondwanaland in the Cretaceous, with the 150 km inflection point on the gradient being the top of the sheared, low-velocity zone at that time. Inflected geothermal gradients have been proposed within the upper mantle beneath other parts of the South African Shield, the North American Craton and the Siberian Shield, based on granular and deformed peridotite suites from Jagersfontein⁶; Louwrencia⁷, SW Africa; Black Butte diatreme, Montana⁸; Ming Bar diatreme, Wyoming⁹; and the Udachnaya diatreme, Yakutia¹⁰. These data cannot, however, be accepted unreservedly as

typical of events within the upper mantle at the time of the generation and ascent of the kimberlite magma. There is evidence to the contrary in xenolith suites from at least three different kimberlite intrusions.

The peridotites and pyroxenites from the Matsoku kimberlite diatreme, Lesotho, have been more thoroughly investigated than any other xenolith suite¹¹⁻¹³. They show a wide variety of textures, including those falling into the 'sheared' class of Nixon and Boyd³. But no matter what degree of deformation the Matsoku xenoliths have undergone, they all apparently equilibrated under the same pressure/temperature (PT) conditions and there is thus no correlation between deformation and increased temperature of equilibration in this particular xenolith suite. The restricted PT conditions indicated are those of Boyd's¹ coarse granular suite. As the Matsoku kimberlite is closely contemporaneous with the Thaba Putsoa kimberlite, and is only 20 km from it, it is difficult to accept a hypothesis involving large-scale horizontal movements in the upper mantle that did not have similar effects at both localities.

In the case of the xenolith suite from the Frank Smith Mine, north of Kimberley, a variety of deformation textures can be found in rocks apparently derived from a restricted mantle zone¹⁴, although the range does not include granular textures as it does at Matsoku, and the equilibration temperatures are close to 1,250 °C.

We have been investigating a suite of xenoliths from the Bultfontein kimberlite diatreme, Kimberley. The garnet lherzolites show a greater textural range than those from Thaba Putsoa and we have recognised a new textural type that indicates a greater degree of deformation than recognised previously in kimberlite xenoliths. This new texture, which may be regarded as a more deformed extension of Bouillier and Nicolas' fluidal mosaic texture¹⁵, comprises recrystallised orthopyroxene neoblasts strung out into bands that alternate with bands of fine-grained olivine neoblasts; furthermore, garnet unaffected by lower degrees of deformation has been disrupted and strung out into chains of small crystals. We call this texture 'banded and disrupted' (BAD).

We have analysed the phases in 26 selected garnet lherzolites from Bultfontein, covering the range of textural types from granular to BAD. The data will be published elsewhere but Table 1 shows analyses of phases in typical

Fig. 1 Plot of Al₂O₃ (in orthopyroxene) against Ca/Ca+Mg (in clinopyroxene) for xenolith suites from Thaba Putsoa⁵ (open symbols) and Bultfontein (solid symbols). Triangles, minerals in rock of granular texture; circles, minerals in rocks of flaser (Bultfontein) and sheared (Thaba Putsoa) texture. The Bultfontein flaser peridotites exhibit porphyroclastic, mosaic and BAD textures.

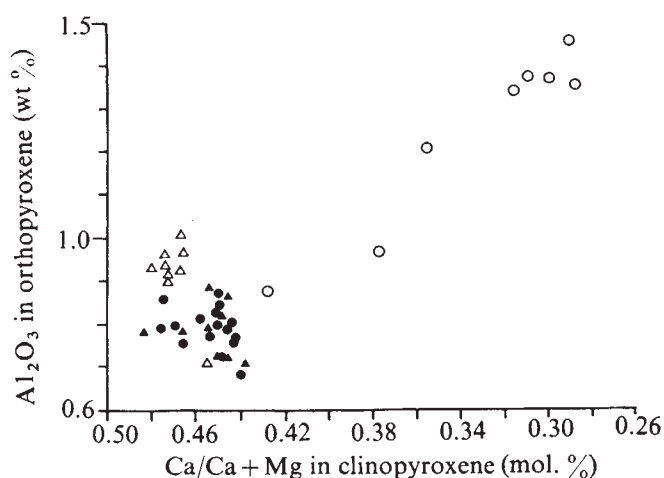


Table 1 Analyses (a) and structural formulae (b) of minerals in typical Bultfontein peridotites at opposite ends of the deformation spectrum

a	BD2382: granular garnet lherzolite				BD2446: banded and disrupted garnet lherzolite			
	Olivine	Orthopyroxene	Clinopyroxene	Garnet	Olivine	Orthopyroxene	Clinopyroxene	Garnet
SiO ₂	40.00	57.38	54.79	41.69	41.51	57.05	54.83	41.93
TiO ₂	0.02	0.04	0.11	0.08	0.00	0.05	0.07	0.05
Al ₂ O ₃	0.01	0.78	2.39	22.62	0.02	0.82	2.51	20.67
Cr ₂ O ₃	0.00	0.06	1.42	2.19	0.02	0.36	2.53	5.01
FeO*	8.87	5.58	2.51	8.19	6.88	4.22	1.88	6.92
MnO	0.04	0.03	0.00	0.36	0.00	0.09	0.07	0.36
MgO	50.96	36.14	16.27	22.62	51.41	36.48	16.54	20.34
CaO	0.02	0.27	21.26	4.78	0.01	0.31	18.94	5.05
Na ₂ O	0.00	0.10	2.00	0.02	0.06	0.09	2.27	0.05
K ₂ O	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.00
Total (wt %)	99.9	100.4	100.7	98.5	99.9	99.5	99.6	100.3
b								
Si	0.979	1.965	1.972	3.022	1.003	1.961	1.982	2.992
Ti	0.000	0.001	0.003	0.004	0.000	0.001	0.002	0.003
Al	0.000	0.031	0.100	1.933	0.001	0.033	0.107	1.732
Cr	0.000	0.002	0.040	0.126	0.000	0.010	0.072	0.282
Fe	0.181	0.160	0.076	0.540	0.139	0.121	0.057	0.413
Mn	0.001	0.001	0.000	0.022	0.000	0.006	0.002	0.022
Mg	1.859	1.845	0.873	1.925	1.852	1.872	0.892	2.164
Ca	0.001	0.010	0.820	0.371	0.000	0.011	0.732	0.386
Na	0.000	0.007	0.140	0.003	0.003	0.006	0.159	0.007
K	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.000
O	4	6	6	12	4	6	6	12
Mg/Mg+Fe	0.911	0.920	0.920	0.781	0.930	0.939	0.939	0.831
Ca/Ca+Mg			0.484				0.451	

*All iron as total FeO.

rocks from the two ends of the deformation spectrum. For the suite as a whole there are three pertinent features. First, no matter what the texture, the Ca/Ca+Mg ratio of the clinopyroxene is in the range 0.422–0.484 (wt %), indicating equilibration temperatures of 950–1,050 °C (ref. 2). Second, the Al₂O₃ content of all the orthopyroxenes is very similar to that in lherzolite orthopyroxenes in the Matsoku kimberlite (0.73–0.89 wt %), again indicating equilibration over a restricted pressure (depth) interval (Fig. 1). In view of the absence of experimental data on orthopyroxenes containing less than 2 wt% Al₂O₃, we are not prepared to draw any pressure (depth) implications from our results, other than to submit that they are of mantle origin, within the garnet lherzolite stability field. Third, the composition of the garnets is broadly similar in most lherzolites, with the exception that in a very few of the more deformed lherzolites, there are small increases in the TiO₂ content.

These data provide evidence that increased deformation cannot always be correlated with significant increases in temperature of equilibration or with increased depth or origin, as is the case for Thaba Putsoa xenoliths. Further, we have observed deformation gradients from granular to BAD textures within the same hand specimens over a distance of 2–3 cm and must assume that the deformation may be both very localised and very variable. This is not consistent with a major tectonic boundary, and may be caused by variable hydrolitic weakening.

It is clear that within the xenolith suite from each locality there is evidence of major deformation, consistent with differential movements in the upper mantle. One feature that is not clear is the sense of these movements. Strong lateral movements, accompanying the breakup of Gondwanaland, are proposed in the Thaba Putsoa model of Nixon and Boyd⁵, whereas the model for the origin of kimberlites by diapiric upwelling in the upper mantle¹⁶, invokes strong vertical movements. The evidence of individuality in the range of textures and equilibration conditions of xenolith suites from different diatremes is possibly more consistent with the latter hypothesis in that it links the characteristics of a xenolith suite with the particular upper mantle movements that culminate in the

emplacement of the kimberlite intrusions. Nonetheless, we do not exclude the possibility that some of the textures developed during the kimberlite event may be additional to textures developed during earlier upper mantle creep. A model involving vertical movement finds support in the geological evidence in that, during the Cretaceous, when most South African kimberlites were emplaced, the South African continent was subjected to strong vertical movements, resulting in peripheral faults downthrowing 18,000 m towards the contiguous ocean basins¹⁷.

We acknowledge the financial assistance of the South African Council for Scientific and Industrial Research and the Anglo-American Corporation of South Africa. The Anglo-American Corporation assisted with sampling and provided analytical facilities. We also acknowledge the interest and advice of J. B. Hawthorne.

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Morarian Orogeny and Grenville Belt in Britain

Now that the nature of the Morarian orogenesis (about 730 Myr BP) is becoming more apparent^{1,2} it seems appropriate to assess its significance in terms of late Proterozoic history of the northern Atlantic region. It has been argued³ that the event is related to subduction, with a suture partly coinciding with the Great Glen Fault. The event has also been associated with the closing of a Precambrian ocean during the time represented by the unconformity between the Stoer and Torridon groups⁴. The Proto-Atlantic opened after the deposition of the Torridon Group. In establishing the orogenic character of the Morarian Event several features are important. First, the event is known to have affected Moine rocks in a zone some 20 km wide. If, as seems likely, the Morarian fabric can be traced as far south as the Great Glen Fault then the belt is some 50 km wide after the Caledonian shortening. Until more is known about the geology of Sutherland, however, it is uncertain whether the entire length of the Moine outcrop (about 250 km) is involved. There is no reason to believe that the Morarian fabric is present to the south of the Great Glen Fault: the likelihood is that the Central Highland Granulites (often named the Moines nowadays) represent post-Moine sediments, but the possibility that they are Moines that were not influenced by the Morarian Orogeny cannot be excluded. Until this problem is resolved it would be prudent to revive the name Central Highlands Granulite for the sub-Dalradian rocks occurring to the south of the Great Glen Fault.

Second, the Morarian belt is an Alpine-type belt. At Glenelg, Lewisian basement has been tightly folded with the Moine cover. Comparable structures may be present in Sutherland where sheets (nappes) of Lewisian gneiss mark a continuation of the Sgurr Beag Slide⁵. In contrast to the Sgurr Beag Slide the tectonic features responsible for these sheets are marked by a substantial number of ultrabasic bodies (serpentines, scyllites, garnet-pyroxene rocks)⁶, which have penetrated the Lewisian and the surrounding Moines. These bodies are, therefore, synchronous with or later than the emplacement of the sheets. There is some evidence that the serpentines antedate the D2 deformation (Caledonian)⁷. It is conceivable that the Lewisian nappes in Sutherland belong to the Morarian Orogeny.

Third the Morarian Belt is asymmetrical. The vergence of the Morarian antiforms is eastwards² in contrast to the westerly translation of the Caledonian structures in north-western Scotland. The 'root zone' of the Morarian antiforms is exposed in the Glenelg region where the western part of the zone has been truncated by the Moine Thrust. If the Lewisian sheets in Sutherland are Morarian nappes rooted to the west then the Moine Thrust in northern Scotland has cut off the root zone. The implication is that the root zone lies wholly or in part beneath the Moine Thrust.

Fourth, the Morarian Orogeny was associated with upper amphibolite facies metamorphism resulting in the development of migmatites on a regional scale. In spite of the apparent lack of igneous rock which can be related to the Morarian Event there are some grounds for regarding the Morarian as an orthotectonic belt.

Fifth, there is no obvious connection between the Morarian Orogeny and the Stoer and Torridon groups which seem to antedate it. Thus, neither of these groups can represent molasse and, setting aside the possibility that the Central Highland Granulites is such, there is no identifiable sedimentary deposit that can be linked with any uplift of the Morarian Orogen.

These considerations allow speculation about a plate-tectonic model in the context of the late Proterozoic history of the northern Atlantic region. Garson and Plant³ have inferred the presence of a subduction zone, active about 730 Myr BP, following the ultrabasic zone in Sutherland and along the line of the later Great Glen Fault. That hypothesis encounters, however, several difficulties⁷. The ultrabasic bodies are emplaced at a high level in the structural pile: they must have penetrated some 2–3 km of Moine rocks to reach their present position. Accordingly, I interpret them as high level diapirs, derived from oceanic lithosphere, which, of course, are not situated on a subduction zone. The geographical association of these bodies with Lewisian sheets is probably significant and suggests a tectonic control (a zone of sliding) for their emplacement. Another possible difficulty is that the model devised by Garson and Plant³ implies that the "Moine" and Dalradian rocks of the Grampian Highlands were deposited on oceanic crust situated to the south of the proposed subduction zone.

As an alternative hypothesis, I suggest that the Morarian suture is hidden beneath the Caledonian nappes, that is, the Glenelg and Rossshire nappes. This follows from the vergence of, and the westerly root for, the Morarian nappes. A further prospect is that the Morarian is a collision-type belt, and the implication of that (see ref. 8) would be that the Moines were deposited on one continent (the Baltic Shield or a microcontinent) and the Stoer and Torridon groups were deposited on another—the Laurentian.

If the portrayal of the Morarian as an orogenic event is correct then it is worth considering its setting in the wider context. On present evidence the event seems to have been localised: apart from in Spitzbergen⁹ and western Ireland¹⁰ there is no firm evidence from the northern Atlantic Region of orogenic activity at 800–700 Myr BP. It may be that the Morarian Event is commonly obscured, to an even greater degree than it is in Scotland, by strong Caledonian overprinting and in this respect anomalous radiometric dates, such as the 700–800 Myr dates recorded in the Appalachians¹¹, are worth re-examination.

But assuming that the Morarian event was localised, the likely hypothesis is that it resulted from the closure of a small ocean basin before the episode of rifting that formed the later Proto-Atlantic⁴. Another possibility is that the Morarian Event represents a delayed closure of the Grenville Ocean in the region of Britain, thus forming the missing link in Britain between Canada and Scandinavia^{12,13}. Orogenic events took place at widely different times during the closure of the Proto-Atlantic and adjacent oceans, and incomplete suturing produced the Erian, Acadian and Taconic episodes⁴. Though the time span between the Grenville and the Morarian events is substantially greater than that separating the Taconic and Acadian episodes of the Caledonian Orogeny, it should be borne in mind that the dated Morarian pegmatites may only date the end of the Morarian Orogenic Cycle.

I thank Drs A. L. Harris and A. D. Stewart for their comments.

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Received May 19; accepted August 18, 1975.

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Magnetic properties of oceanic pillow basalts from Macquarie Island

WE summarise here the results of a combined palaeomagnetic, hysteresis, thermomagnetic, and electron microprobe analysis of late Tertiary oceanic pillow basalts from Macquarie Island, which indicate that pillow basalts from a significant depth in the oceanic lithosphere have suffered burial metamorphism during the crustal accretion process. This metamorphism results in an intensity of natural remanent magnetisation (NRM) of $\sim 10^{-3}$ gauss, a substantial component of viscous remanent magnetisation (VRM), and an order of magnitude decrease in the Koenigsberger ratio relative to unaltered pillow basalts. Since a pillow lava layer with such a low NRM intensity is insufficient to account for the observed amplitude of marine magnetic anomalies, we conclude that in the case of Macquarie Island the underlying basaltic and doleritic dyke complex must also contribute to the observed anomalies.

Our data suggest a model in which the contribution to magnetic anomalies by the pillow basalt layer decreases with the age of the oceanic crust, though the contribution from the underlying dyke complex may not be susceptible to decay¹. The data also suggest that the credibility of the pillow basalt layer as the entire source of marine magnetic anomalies in old oceanic crust (at least 30 Myr BP) must be re-examined.

Field geological, petrological, geochemical, and marine geophysical data indicate that Macquarie Island (54° 15'S, 159°E) is an uplifted segment of late Tertiary (~ 30 Myr old) oceanic crust which was formed by seafloor spreading of the Australia–Antarctic Ridge^{2,3}. Pillow lavas exposed in the North Head region of Macquarie Island are relatively fresh and have not undergone burial metamorphism; however, pillow basalts exposed near Langdon Point have undergone significant burial metamorphism. Varne and Rubenach² have argued that this metamorphism is characteristic of the crustal accretion process at spreading oceanic ridges and is not the result of a metamorphic event associated with the tectonic uplift of Macquarie Island. This evidence indicates that the North Head exposures are representative of the upper (100–200 m?) portion of the oceanic basalt layer, whereas the Langdon Point lavas are from deeper oceanic crust ($> 1/2$ km deep?). To investigate the effects of burial metamorphism and seafloor weathering, oriented core samples were collected at 4 sites (nos 8–11) near Langdon Point and at 4 sites (nos 12–15) on the eastern coast of North Head.

Because of tectonic disturbance, NRM directions cannot be used for palaeomagnetic pole determinations but are very

useful in determining the presence of stable and viscous remanence at the sampling localities. The important mean parameters of natural remanence observed at both Macquarie Island sampling localities are summarised in Table 1. Directions of NRM for the Langdon Point sites show streaking toward the present direction of the axial dipole field at the sampling locality. On alternating field (a.f.) demagnetisation, the directions move away from the axial dipole direction. These observations indicate a significant component of viscous remanent magnetisation (VRM) in these metamorphosed pillow lavas. Experiments of the VRM acquisition confirmed the indication of a large viscosity coefficient, S , in samples from this locality. Directions of natural remanence at North Head sites show only a small tendency to streak towards the axial dipole field. Experiments on the acquisition of a.f. demagnetisation and acquisition of VRM also indicate that the unmetamorphosed basalts from North Head are not susceptible to large components of viscous remanence.

The geometric mean intensity of the NRM at both Macquarie Island sampling localities is surprisingly low. The geometric mean intensity is 0.69×10^{-3} gauss for samples from North Head and 0.57×10^{-3} gauss for Langdon Point samples (Table 1). In contrast, NRM intensities of some dredged samples have exceeded 10^{-1} gauss, and deep-tow magnetometer profiles over oceanic ridges also indicate a strongly magnetic layer of pillow lava in young oceanic crust^{4,5}. If the NRM intensity of these young pillow basalts does not decrease with depth into the oceanic crust, a thin (< 1 km thick) layer of strongly magnetic pillow basalts could account for the entire amplitude of marine magnetic anomalies. Seafloor weathering, however, causes low temperature oxidation of the magnetic minerals resulting in a decreased intensity of NRM^{6,7}. Data collected during the Deep Sea Drilling Project (DSDP) indicates that the upper pillow basalts of ancient oceanic crust have NRM intensities of $\sim 10^{-3}$ gauss^{8,9}. These values are in close agreement with NRM intensities of Macquarie Island pillow basalts, and are, perhaps, typical of old (> 30 Myr) oceanic crust in general. If that is the case, then the contribution of the pillow basalt layer alone is inadequate to account for magnetic anomalies observed over old oceanic crust.

From measurements of low field susceptibility, χ , the Koenigsberger ratio, Q ($= \text{NRM}/(\chi)(0.65 \text{ oersted})$), was determined for each sample. The mean Q value for North Head samples is similar to that observed in Atlantic DSDP basalts. The Langdon Point specimens show, however, a very high susceptibility and, consequently, a low Koenigsberger ratio. This high χ and low Q for Langdon Point samples indicates a difference in magnetic mineralogy between these metamorphosed pillow basalts and the unmetamorphosed pillows from North Head. Hysteresis properties for North Head samples indicate a distribution of magnetic grains which is predominantly within the single-domain range (R. F. B., S. K. B. and J. H. S., unpublished). By contrast, the hysteresis properties for Langdon Point samples indicate a substantial multi-domain (MD) content. This higher MD content would explain the higher magnetic viscosity coefficients in the Langdon Point samples.

Results of thermomagnetic analyses also indicate an important difference in magnetic mineralogy between the two Macquarie Island sampling localities. Irreversible thermo-

Table 1 Average parameters of remanent magnetism

Locality	Natural remanence (10^{-3} gauss)	Low field susceptibility (10^{-3} gauss oersted ⁻¹)	Koenigsberger ratio	Viscosity coefficient (10^{-5} gauss oersted ⁻¹)*
Langdon point (sites 8–11)	0.57	2.80	0.31	4.3
North Head (sites 12–15)	0.68	0.11	9.8	1.2

*Time measured in minutes.

magnetic curves were observed for all samples from North Head. An initial Curie temperature of between 300 and 400 °C was followed by an increase in magnetisation and a subsequent final Curie point between 500 and 580 °C. The saturation magnetisation after thermal cycling was always greater than the initial saturation magnetisation. This type of irreversible thermomagnetic behaviour has been observed in dredged pillow lavas⁷ and in DSDP basalts^{8,9}. The thermomagnetic results—and microprobe examinations of the opaque minerals (R. F. B., S. K. B. and J. H. S., unpublished)—indicate that the magnetic minerals in the North Head samples are non-stoichiometric titanomagnhaemite. These cation-deficient phases are the products of low temperature oxidation resulting from prolonged seafloor weathering of the late Tertiary oceanic pillow basalts.

All of the Langdon Point samples show reversible thermomagnetic curves with a single Curie temperature very near 580 °C, indicating that the magnetic mineral in these metamorphosed pillow lavas is magnetite containing little or no titanium. Reversible thermomagnetic curves indicating Curie points of 580 °C have not been observed previously in analyses of dredged pillow basalts or DSDP basalts. This 'unusual' behaviour is almost certainly the result of burial metamorphism undergone by the Langdon Point basalts. The fine-grained titanomagnetites in young dredged pillow basalts are known to be very susceptible to chemical change when heated. At any significant depth in the oceanic crust pillow basalts would be exposed to heating by the intrusion of feeder dykes leading to overlying pillow lavas, the emplacement of underlying sheeted dyke and plutonic complexes, or even by exothermic hydration reactions within the metamorphosed pillow lavas themselves². Thus, the metamorphosed pillow lavas from Langdon Point and their resulting magnetic properties are thought to be the natural consequence of burial metamorphism experienced during seafloor spreading by basalts at significant ($> 1/2$ km?) depths in the oceanic crust.

The evidence from DSDP basalts, and the data presented here on pillow lavas from the North Head region of Macquarie Island indicate that the progressive submarine weathering of the oceanic crust decreases the NRM intensity of the upper portion of oceanic crust to $\sim 10^{-3}$ gauss. Lowrie, *et al.*⁸ have suggested that the 10^{-3} gauss NRM intensities of DSDP basalts are perhaps only representative of the strongly weathered zone at the top of the pillow lava layer and that unweathered pillow lavas below the weathered zone may be more strongly magnetised. But if the pillow lavas from Langdon Point on Macquarie Island are representative of pillow basalts from depths of 500–1000 m, the NRM intensity of these deeper extrusives would also be of the order of 10^{-3} gauss. Since a 1-km thick layer of pillow basalts magnetised at 10^{-3} gauss is not sufficient to yield the observed amplitude of marine magnetic anomalies, we conclude that the underlying basaltic and doleritic sheeted dyke complexes also contribute to the anomalies. Detailed analysis of changes in marine magnetic anomaly profiles with age of the underlying oceanic crust has led to a similar conclusion (R. J. Blakely, unpublished).

We thank Dr R. I. Garrod, Director of Antarctic Division, Australian Department of Science, and Dr R. Varne for assistance throughout this project. Assistance during field work was given by P. Williamson and P. J. Gloag; many of the sample measurements were done by Dave Bodgan and Jim Mellema. This project was funded by the National Science Foundation.

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Effect of oxygen on aldolisation reactions

ALTHOUGH the kinetics of aldolisation reactions have been studied^{1,2}, no observations have been reported concerning the effect of oxygen on their rates and kinetics. We have studied polarographically the aldolisation of acetaldehyde in 1 M lithium hydroxide in the absence of oxygen and spectrophotometrically in the presence of air using absorbance at 230 and 277 nm. A difference in the concentration–time curves obtained using these two procedures was observed.

The base-catalysed autoxidation of ketones is probably³ initiated by the interaction of molecular oxygen with carbanions formed in alkaline media and in the past the observed discrepancy has, therefore, been attributed to the interaction of atmospheric oxygen with carbanions formed as intermediates in the course of aldolisation.

To test this hypothesis, we studied reactions of α -phenyl-substituted aldehydes of the type $\text{RCH}(\text{C}_6\text{H}_5)\text{CHO}$ in alkaline media. The pK values of these compounds⁴ are such that they exist in 1 M lithium hydroxide almost completely in the carbanion form. Observations of these compounds during kinetic runs showed that they behaved differently in the presence and absence of oxygen. In the absence of oxygen, polarographic and spectrophotometric measurements gave identical rates. As a side product of the reactions RCOC_6H_5 compounds are formed in the presence of air⁵, further confirming the reaction of carbanion with oxygen. The latter reaction has also been postulated as a side product of the oxidation of phenylacetaldehyde to benzoic acid⁶ and in association with the alkaline rearrangement of aconitine⁷.

Thus some discrepancies reported for the kinetics of aldolisation may result from an interaction between molecular oxygen and the carbanion intermediate. Consequently, in future studies of aldolisation and other reactions which involve carbanion formation, oxygen should be excluded completely.

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Polymer drag reduction in Taylor vortices

THE Toms effect¹, whereby the addition of small quantities of polymer can typically produce 50% reduction in drag, occurs in turbulent flow systems. We report preliminary observations of a similar drag reduction behaviour observed in a Couette apparatus when toroidal Taylor vortices² are generated by rotating the inner of two concentric cylinders at sufficient speed; in this case the flow field is well understood and does not vary with time.

Simple shear alone as generated by normal Couette flow has little effect on deforming flexible polymer molecules and can be expected to exhibit no drag reducing effect; however, extensional flows such as pure shear, axial extension and compression do have the ability to extend molecules and the extensional viscosity when polymers are present may be orders of magnitude greater than the shear viscosity³. If this class of flow is present significant effects can be expected when polymers are added. In Taylor vortices the existence of extensional flow regions between each counter rotating vortex has already been

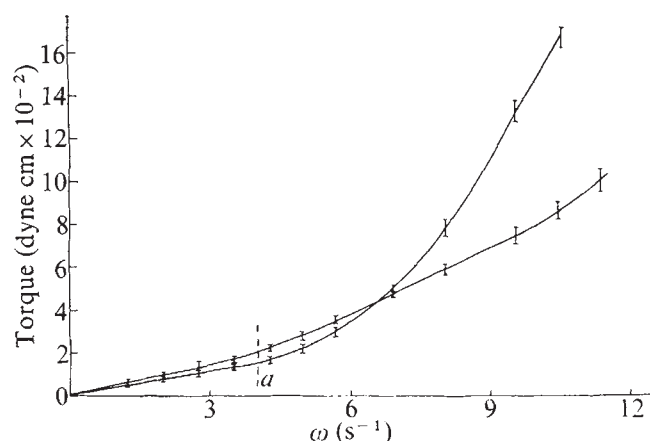


Fig. 1 Graph of torque as a function of angular velocity for a Couette apparatus 30 cm long, of inner cylinder diameter 2.0 cm and gap width 0.37 cm.

identified⁴ as the cause of nucleation of extended chain polymer crystals.

With a Couette apparatus we have measured the torque on the stationary outer cylinder using a fine torsion wire suspension. Figure 1 shows the torque measured for different rates of rotation of the inner cylinder using both water and polymer doped solutions. The polymer was polyethylene oxide (Union Carbide WSR301) of molecular weight $M_w \approx 4 \times 10^6$. The solution was renewed before each series of measurements to minimise the effect of possible shear induced degradation. For a low angular velocity of the inner cylinder when the flow is simple shear, the torque against ω curve obeys an essentially linear relationship for both solutions where the gradient of the line gives the shear viscosity, which is about 20% higher for the 50 p.p.m. polyethylene oxide (WSR301) water solution. At a critical speed (a in Fig. 1) the torque starts to increase non-linearly. This speed corresponds well with that predicted by Taylor² at which the secondary Taylor vortex pattern develops. Above this critical speed we assume that subsequent differences in the measured torque curve from the extrapolated straight line represent the additional drag caused by the secondary Taylor vortex flow. When Taylor vortices are well developed we see that the drag as measured by the torque is significantly reduced when polymer is present.

From our torque measurements we cannot say with precision

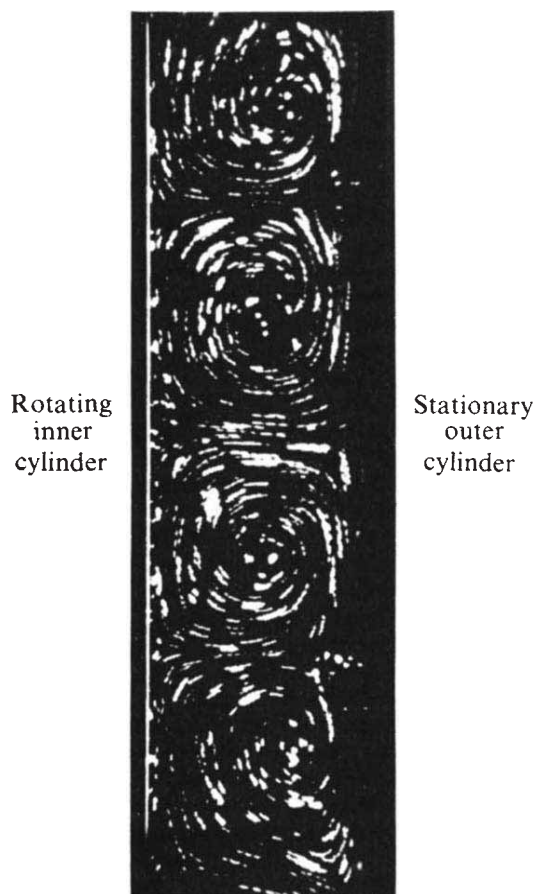
if there is a change in the onset of instability with the addition of polymer; however, our results do indicate that this change if present is not large. Changes in the onset of instability have been examined in detail by others⁵⁻⁸, however, these studies were confined to rates of rotation close to the critical region (a in Fig. 1). The large drag reducing effects we are concerned with, occur at greater rates of rotation well removed from this critical region.

By illuminating the gap between the two cylinders with a plane beam of light directed vertically along the radius and passing through the glass outer cylinder, the nature of the Taylor vortices could be examined in profile by observing scattered light from small tracer particles put into the solution.

Streamlines of the vortices observed at right angles to the incident beam are shown in Fig. 2. No detectable difference could be found in their profile with the addition of polymer, but examination of velocities using short time exposure photographs indicated that the speed of the vortices measured in a region where the vortices meet midway in the gap between cylinders, increased by up to 10% at $\omega = 13 \text{ s}^{-1}$ with the addition of polymer. This result is contrary to the expectations of some theories⁹ of drag reduction and indicates that drag reduction in Taylor vortices does not occur by suppression of vorticity; indeed the horizontal components of vorticity as represented by the secondary flow pattern of our experiments is enhanced.

We have shown that addition of polymer does not change significantly the onset of the secondary flow or suppress its vorticity and thus other mechanisms must be explored to explain the origin of the effect. We believe chain extension occurs near the cylinder walls in regions of compression where the vortex flow approaches either inner or outer wall. The possibility of

Fig. 2 Photographic profile view of Taylor vortex pattern generated in Couette apparatus; inner cylinder diameter 4 cm and gap width 1.0 cm.



chain extension occurring in the regions of extension where the vortices move away from the walls is not thought likely because related experiments firing a jet of polyethylene oxide solution at right angles to a flat surface showed localised birefringence corresponding to chain extension, near and parallel to the wall for jet outflow (axial compression) but no visible birefringence for the reverse flow direction when fluid was sucked into the jet (axial extension). We therefore conclude that in regions of compression in which chain extension occurs the extensional viscosity can be expected to increase significantly. As no drag reducing mechanism can be seen in the vertical plane we conclude that either the primary or secondary flow must be modified in the horizontal plane in such a way as to cause the reduction in overall momentum transfer to the outer wall. Further velocity profile and additional birefringence experiments viewing the horizontal plane of the Taylor vortex flow system will be carried out to establish this point.

At this stage certain generalisations concerning conditions for drag reduction may be proposed: an elongational flow system is required to produce chain extension; and the chain extension thus achieved should then be capable of reducing the momentum transfer (which is the cause of the drag) in a manner not specified in detail at the moment. This reduction of momentum transfer, however should not eliminate the elongational flow field which has produced the chain extension in the first place; its continued survival is a prerequisite for the whole effect.

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Acoustic Bragg diffraction from human tissues

ANALYTICAL methods based on the use of ultrasonic beams are potentially of interest for the remote, *in vivo* characterisation of the mechanical structure of human tissues^{1,2}. We have investigated, from this point of view, the relationship, for a specific volume of tissue, between the recorded ultrasonic backscattering amplitude and the orientation of the tissue structure to the ultrasonic beam. Experimentally this measurement is closely analogous to Bragg's X-ray crystallography arrangement except that we use 180° backscattering geometry.

Our early observations suggested qualitatively that the acoustic Bragg traces obtained were indeed characteristic of the particular tissues examined³ and modelling calculations have shown that the data are consistent with the hypothesis⁴ that diffraction arises predominantly from the connective tissue microstructure of the organs involved, for example the lobular network in liver tissue⁵. Here we present quantitative evidence that the method can provide objective characterisation of tissue structure.

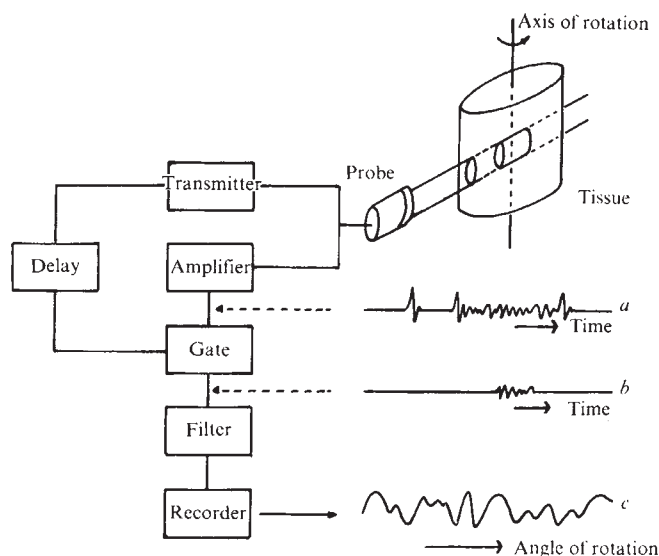
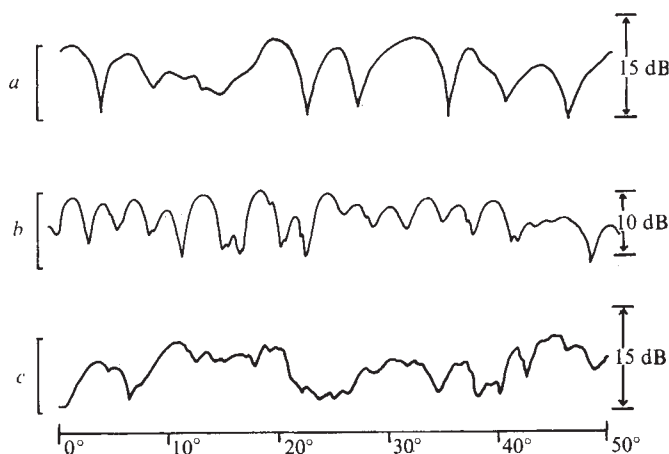


Fig. 1 Schematic diagram of the experimental arrangement for investigation of the backscattered Bragg diffraction characteristics of human tissues: a, received echo train from tissue specimens; b, gated output of received echo train; c, diffraction pattern obtained by frequency filtering the gated output while rotating the specimen.

Experimentally the apparatus is that of a conventional ultrasonic pulse-echo system (Fig. 1) in which an approximately cylindrical tissue specimen is positioned in a water-filled sound tank so as to lie directly in the sound beam, its long axis being normal to the direction of the beam. The sample is then rotated about this axis to enable backscattering amplitude measurements to be made from all angles. A time gate centred on this axis of rotation selects a portion of the returning echo train such that the total received echo amplitude corresponds to the backscattering from a specific volume of tissue, determined by the beam width, pulse length and appropriate time gate. Following frequency filtering of the returned echo train, plots of echo amplitude as a function of angle of rotation are obtained for one complete rotation. Some limited examples (0-50°) are shown in Fig. 2. An interesting feature of these diffraction patterns is that they seem to be characteristic of the particular tissue and presumably reflect corresponding differences between the respective tissue structures.

Evidently, several possible analytical approaches could be taken towards establishing the significance of differences or similarities between such traces and we report here the results of one of these, in which we have computed, by the fast Fourier transform method, the distributions of angular frequency

Fig. 2 Observed Bragg diffraction patterns from three types of human soft tissue, examined at 1.0 MHz. a, Liver; b, brain; c, spleen.



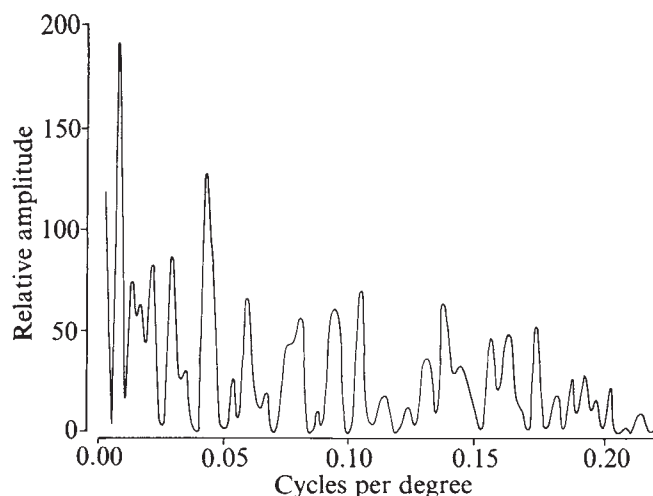


Fig. 3 Power spectra derived from the Bragg diffraction record of a specimen of human liver tissue, examined at 1.0 MHz.

components contained in the traces. A typical power spectrum obtained by this method from a single 'acoustic Bragg trace' has a somewhat complex structure, part of which may correspond to the 'picket-fence effect'⁶ inherent in the fast Fourier transform procedure (Fig. 3). For the purpose of investigating possible systematic differences between data from different tissues we have, therefore, smoothed the raw data in each spectrum by taking a seven-point average. Means of the smoothed power spectra have then been computed for each of the three sets of tissue specimens and these are plotted, together with their corresponding standard errors, in Figure 4.

These results demonstrate that this diffraction phenomenon observed in our initial investigations can be quantitatively related to the specific structure of the type of tissue. Although this finding is reasonable in view of the well known differences in the microscopic appearances of tissues of different types, its complete understanding in terms of the mechanical structure, including factors such as the existence of degrees of short range structural order, must be a subject for further research. Meanwhile, it suggests the prospect of a non-invasive diagnostic technique for distinguishing between different tissue structures *in vivo*, and thus depicting any pathological changes within a tissue region of diagnostic concern. An instrument designed to carry out such an *in vivo* investigation in the human liver has recently been constructed and will be described elsewhere.

We thank J. Milan for advice and assistance on various

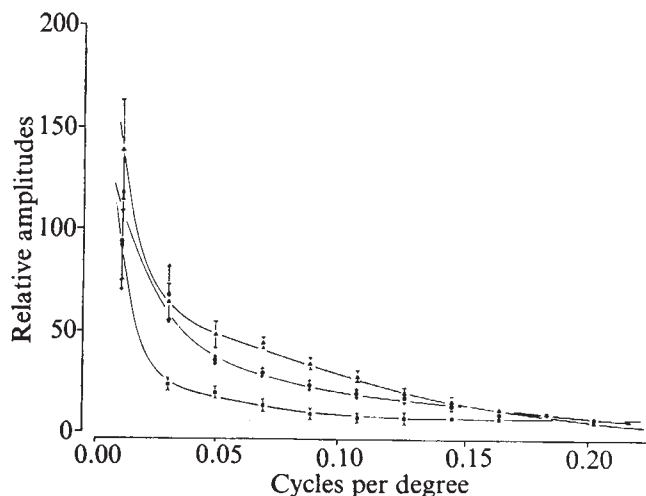


Fig. 4 Mean power spectra from three types of human soft tissue, each derived from the smoothed power spectra of twelve similar tissues examined at 1.0 MHz (▲, liver; ●, spleen; ■, brain).

aspects of computing techniques. D. N. was supported by a grant from the Medical Research Council.

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Effect on mortality of the 1974 fuel crisis

THE 1974 fuel crisis was a natural experiment. It presented the opportunity to test the hypothesis that a decrease in vehicular exhaust fumes would have a beneficial effect on health. During the first quarter of 1974 retail gasoline sales were reduced by 9.5% in San Francisco and Alameda Counties, California. Total exhaust emissions were reduced by an even greater amount because of hoarding of fuel and lowered highway speed limits. To assess the possible effects of this selective decrease in pollution, mortality rates from these two counties were examined. The rates for the first quarter of 1974 were compared with corresponding rates from the first quarters of 1970-73.

Table 1 First-quarter mortality rates per 100,000 population in two California counties, 1970-74

	San Francisco County		
	1970-73	1974	% Decrease
All causes*	377.5	327.0	13.4
Cardiovascular disease†	128.8	107.3	16.7
Chronic lung disease‡	7.3	4.9	32.9
	Alameda County		
	1970-73	1974	% Decrease
All causes*	227.1	209.7	7.7
Cardiovascular disease†	84.2	74.8	11.2
Chronic lung disease	5.0	3.1	38.0

* Excluding all deaths from vehicular accidents.

† For reasons of data availability, this category includes acute myocardial infarction and chronic ischaemic and arteriosclerotic heart disease in Alameda County and all cardiac deaths in San Francisco County.

‡ Includes chronic bronchitis, asthma and emphysema.

Dramatic decreases were noted in death rates for several major categories of disease (Table 1). These included all causes combined (with vehicular accident deaths excluded); pooled cardiovascular deaths; and deaths from asthma, chronic bronchitis and emphysema (denoted as "chronic lung disease"). Rates for the comparison years have been combined for simplicity of presentation. It should be noted, however, that the rates for those years were rather homogeneous. For the disease categories studied, the 1974 first-quarter rates were lower than any of the 1970-73 first-quarter rates. The 13.4% decrease in all-cause mortality in San Francisco, the more urbanised county, was almost twice that in Alameda County (7.7%). The disease category showing the greatest relative change was chronic lung disease.

In general, when the data were analysed by age, the greatest change occurred in the under 45 age group, and the least change among those over 65. We believe that this may be a result of differential exposure. Both sexes showed decreased mortality.

There was no first-quarter secular trend during the period

1970–73 for those disease categories examined. Consideration of the time trend for all quarters in San Francisco revealed a relatively repetitious and predictable seasonal variation with sharp upward swings in winter quarter death rates. During the period January 1, 1969–March 31, 1974, this cyclic trend was interrupted only by the sharp decline in mortality during the first quarter of 1974. Analysis of weather and air stability data, relevant pollutant levels and the pattern of influenza and pneumonia deaths (sometimes thought to influence cardio-respiratory deaths) support the hypothesis that a decrease in vehicular exhaust fumes would have a beneficial effect on health.

It is important that these data be analysed further and in more detail. Such analyses will include comparison of mortality rates in urban–rural subdivisions of Alameda County, consideration of the seasonal and secular trend in Alameda County for the period 1970–74, examination of several other causes of death (particularly those causes not expected to be affected by air pollution) as well as analysis of mortality data in the period following the crisis.

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Sweeping flight and soaring by albatrosses

ALBATROSSES can fly over the sea for a long time without flapping their wings. Raleigh¹ worked out a suitable method, known as dynamic soaring, to account for this and Idrac² showed that this was the method used by the birds.

Dynamic soaring makes use of the gradient in wind velocity near the surface of the sea. The bird gains energy by climbing into the wind: as it climbs it loses forward speed, but it gains airspeed by rising into the faster winds at the higher levels. It can continue to climb for as long as the wind velocity gradient is sufficient. Similarly, the bird can gain energy by losing height downwind. Combining these manoeuvres it is able to maintain flight, climbing upwind and gliding downwind in a series of swoops³.

I have suggested⁴ another method which could be used by albatrosses either in combination with dynamic soaring or as an alternative to it. Here I present calculations and a computer simulation showing that the method can provide enough energy for sustained flight.

Albatrosses often fly along the flanks of waves. This was observed by Idrac², who did not attribute any importance to it. He remarked elsewhere that he had found rising air currents directly related to the waves, in some cases greater than 2 m s^{-1} at 8 m above the sea and still detectable at 15 m. He did not think that the albatross made any use of these variations in the airflow except in exceptional cases, but they would be available for the method of soaring proposed here. It has been proposed that the birds do make incidental use of these updrafts. Pennycuik⁶ writes that "the birds use 'static' soaring along waves combined with dynamic soaring in a rather intricate way". Jameson⁷ says more specifically that the birds may use "the strong updrafts near the crests of the wave to gain considerable height—an initial kick-off, as it were, of 10 or 15 feet . . . the bird making only incidental use of the rising air currents close above the waves . . ."

The method which I propose is this static soaring to provide the initial 'kick-off', but by specifying the method more accurately it can be seen that it is more powerful than had been suspected.

The bird would take off from the crest of a wave and position itself in the rising current against the flank of the wave, maintaining this position in gliding flight. In still air, it would lose height, but in air which is rising over the wave it could maintain height by slope-soaring or could even gain height. Alternatively, it could use the upcurrent to gain speed. It could sweep along the flank of the wave, staying in the strongest upcurrents close to the surface. When the speed is sufficient, or when the bird reaches the end of the wave, it could use the speed by soaring upwards. It could then glide freely in any direction, losing height gradually until it again become necessary to swoop down and fly against the flank of a wave. Thus flight could be maintained indefinitely. This method of soaring, I shall call sweeping flight.

The height to which a bird can zoom out of slope lift can be calculated if the flight polar of the bird and the strength of the lift are known. The flight polar expresses the relationship between the flying speed V of the bird and its sinking speed S in still air. Usually, as flying speed increases the sinking speed will increase at an accelerated rate. A relatively flat polar would express the ability of the bird to fly at high speeds while maintaining a low rate of sink.

Height can be maintained by flying in air which is rising at a rate to match the sinking speed of the bird. If the bird flies close to the water in slope lift (air rising over a wave), it can increase its flying speed until its sinking speed matches the upward velocity of the air. Thus for any given upflow we can read off directly from the polar the maximum speed V_2 which the bird can reach without losing height.

Flying speed can be converted into height by climbing rapidly. If the bird can remain under control down to some minimum gliding speed V_1 , then its excess kinetic energy is given by $E = \frac{1}{2}m(V_2^2 - V_1^2)$, where m is the body of the bird. Neglecting drag, which is relatively small, the whole of this kinetic energy can be converted to potential energy by soaring to a height h . Thus:

$$h = E/mg = (1/2g)(V_2^2 - V_1^2)$$

A greater height can be reached in a wind gradient by climbing against the wind and so making use of dynamic soaring, but I shall now neglect dynamic soaring and consider only the height which can be reached by sweeping flight alone.

A bird with a flat polar will be able to reach a high speed in a relatively light upcurrent, and will be able to climb to a greater height. Birds with a flat polar will be well suited aerodynamically to the method of flight described here, whereas those with a steep polar will not be able to use it at all. This requirement, of a flat polar, is the same as that for dynamic soaring, so that a bird which is suited to one method would be suited to the other and the two methods could easily be used in combination or alternatively. Dynamic soaring could be used when the wind gradient extends up to a suitable height, perhaps 20 m; sweeping flight could be used when winds are strong but the wind gradient is not suitable.

Albatrosses, with their long narrow wings, must have a relatively flat polar, but accurate data are difficult to obtain. Bramwell and Whitfield⁸ give a curve derived from scanty data by Schmitz¹²; this was the polar used in my computer simulation. Wood⁹, for his computer simulation of dynamic soaring, used a standard aerodynamic formula with constants derived from Lanchester's¹⁰ figures for weight and wing measurements of an immature specimen of *Diomedea exulans*. This formula gives a polar closely parallel to that of Bramwell and Whitfield, but with about 0.2 m s^{-1} less sink. Thus, using the Bramwell and Whitfield polar gives a conservative estimate of performance on sweeping flight, as compared with Wood's simulation of dynamic soaring.

For waves of steepness $1/12$ and a wind of 6 m s^{-1} , the upcurrent near the wave is 1.65 m s^{-1} . This is not unrealistic, since Idrac observed even greater upcurrents in association with waves. On the Bramwell and Whitfield polar, 1.65 m s^{-1}

of sink corresponds to a flying speed of 23 m s^{-1} , and it seems that the bird can still fly at speeds of about 12 m s^{-1} . Thus, $h = 19.7 \text{ m}$. This is considerably greater than the 10 or 15 feet suggested by Jameson, and indicates that the method would be a practical one.

To obtain a more realistic estimate of performance, a program was written to simulate the sweep method of soaring on a computer. It was assumed that the wind velocity does not vary with height, and that the flow of air follows the (water) waves at the surface, so producing a sinusoidal vertical component of wind velocity; this component reduces vertically with height, reaching zero at twice the wave height. Performance is monitored by showing wave and bird on a display screen, and the print out shows flying speeds, height reached, cycle period and so on.

The simulation was run for various wind velocities and the results are shown in Fig. 1. Wave height was kept constant at 3 m. (Wave height is relatively unimportant, except in respect of giving space to manoeuvre, since wavelength tends to vary with wave height so leaving wave slope invariant.)

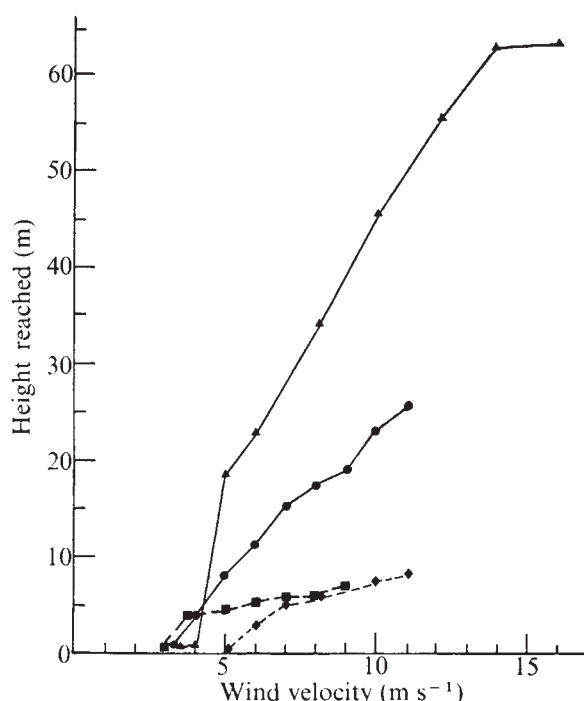


Fig. 1 Performance of four gliders using the method of sweeping flight. ●, Albatross; ■, *Pteranodon*; ♦, fulmar petrel; ▲, glider Ka6CR.

At low wind velocities ($< 3.2 \text{ m s}^{-1}$), the upcurrents are insufficient to sustain even minimum-sink gliding flight during the sweep. The bird stalls and the flight is terminated. With slightly higher wind velocities the sweep can be maintained but flying speed is increased only slightly, since the increased sinking speed of the bird soon matches the upward velocity of the air. In such cases the simulation gives short sweeps punctuated by small hops. At still higher wind velocities, the flying speed can be increased substantially during the sweep and the bird then climbs to a useful height, comparable with heights observed in practice. Maximum height reached is 26 m, with a maximum period of 62 s.

The simulation was also run with polars for the fulmar petrel, the Cretaceous flying reptile *Pteranodon* and a glider. The glider needs far more wind than the albatross to remain airborne, but far outperforms the bird at high wind velocities. The calculation is unrealistic, however, since it ignores the difficulties of placing a large glider between the waves.

The polar for the fulmar petrel *Fulmaris glacialis* was obtained by Pennycuik from measurements using a cine-camera

technique⁵. Though neither the albatross data nor those for *F. glacialis* are claimed to be accurate, it is clear that the fulmar has a greater sink at all speeds of which it is capable, and its polar is much steeper than that of the albatross. The simulation shows the fulmar reaches a maximum height of only 8 m.

The polar for *Pteranodon* was obtained by Bramwell and Whitfield from wind-tunnel measurements of a model⁶. Again, the figures cannot be claimed to be accurate, but provide a working estimate. With this polar, the simulation shows *Pteranodon* as reaching a maximum height of 7 m.

Pennycuik concluded for *F. glacialis*, and Bramwell and Whitfield for *Pteranodon*, that they were primarily slope-soarers and were unsuited to dynamic soaring. They are also far less suited to the suggested method of sweeping flight than is the albatross.

I thank Dr Colin Pennycuik for his comments and suggestions, and John Lazarus for some useful references.

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Localisation of human peptidase-A structural locus from studies on a cultured lymphoblastoid line

LONG term genetic analysis of lymphoblastoid cell lines provides a practical means of studying the types of spontaneous and induced mutation that can occur in human somatic cells^{1,2} and of following the evolution of distinct populations of cells within a bulk culture^{3,4}. Occasionally, data may be expected to emerge which facilitate the localisation of human structural genes. We now report the first such application of this technique.

The human lymphoblastoid cell line F137 (ref. 5) has been maintained in Edinburgh since 1969 and more than 200 clones have been grown (sometimes after treatment of the bulk culture with mutagen) from single cells plated out in individual wells of plastic culture plates^{1,4}. The parent line has been examined at intervals for the electrophoretic pattern of more than 30 isoenzymes and each clone, when it has reached a sufficient cell number (usually within 2 months of isolation) has been analysed in the same way¹. Chromosome preparations have been made from both parent line and clones and analysed after staining with quinacrine dihydrochloride^{6–8}.

The original F137 line was heterozygous at the pep-A locus¹ with the phenotype pep-A-2-1. In an early series of experiments (May 1971), one clone, A56 (ref. 1), seemed to be hemizygous for pep-A-2. Over the next three years, a further 133 clones were isolated from the original line; 14 had the pep-A-2 phenotype, two had an asymmetrical electrophoretic pattern with partial loss of the pep-A-1 band, and the remainder had the original symmetrical pep-A-2-1 pattern¹. Quantitative assay of peptidase-A confirmed that loss of the pep-A-1 electrophoretic band was associated with a 50% reduction in enzyme activity⁴.

Human lymphoblastoid cell lines are not entirely stable with respect to chromosome constitution, and various rearrangements can be detected when cultures are re-examined at intervals over a period of years^{1,3}. The modal karyotype of the F137 line during the period of this study was 47, XY, the extra chromo-



Fig. 1 Chromosome pairs 4, 9 and 18 from quinacrine-stained metaphase spreads representative of the parent line F137 (*a*), clone H₅ (*b*), clones with a 9/18 translocation (*c*) and those with a 4/18 translocation (*d*). Clones with the aberrations shown in *b* and *c* have lost activity of the pep-A-1 allele. The 4/18 translocation (*d*) is not associated with any change in pep-A phenotype.

some being a small submetacentric marker about the size of a chromosome 17/18 but distinguishable from them on quinacrine fluorescence.

The original pep-A-2 clone (A56; ref. 1) was lost before it could be examined cytogenetically. Chromosome analysis has been carried out on all subsequent clones showing alterations in the expression of the pep-A gene and on 84 of the 116 clones isolated over the same period which have retained the symmetrical pep-A-2-1 phenotype. From 4–30 metaphase spreads have been photographed and fully analysed from each clone.

Table 1 and Fig. 1 demonstrate the complete relationship between certain chromosome rearrangements involving chromosome 18 and changes in the pep-A phenotype of individual clones. One pep-A-2 clone (H₅) differed from the modal karyotype of the parent line only in having a deletion at the mid-point of the long arm of one chromosome 18 (Fig. 1*b*), a finding which supports the previous assignment of the human pep-A

Table 1 Pep-A phenotype and aberrations involving chromosome 18 in one hundred clones of F137*

Pep-A phenotype	Aberrations involving chromosome 18			
	No abnormality	4/18 translocation	9/18 translocation	18q—
2-1	59	25	0	0
2	0	0	15†	1

* One isolate with an asymmetrical pep-A-2-1 pattern is excluded as not being a true clone but a mixture of cells with a 9/18 translocation and those with the F137 modal karyotype.

† Includes clone J28 with 10% pep-A-1 activity.

structural locus to the distal half of the long arm of chromosome 18 (refs 9–12).

The other 13 pep-A-2 clones all had an identical translocation (Fig. 1*c*) in which almost the whole of the long arm of one chromosome 9 was fused with the long arm of an 18, the point of fusion being marked by an intercalary band of heterochromatin from the centromere region of the 9 (Figs 1*c* and 3).

None of the 84 pep-A-2-1 clones examined contained any cells with the 9/18 translocation. Thirty-one had a karyotype identical to that of the modal cells of the parent line and 38 showed minor variations, most of which had been recognised in the parent line⁴. A further 25 had the rearrangement shown in Fig. 1*d* (cells of this type were also represented in the parent line in February/March 1973). In this case there was a break at the distal end of the long arm of a number 18 with a translocation on to it of approximately half the long arm of a number 4.

As Fig. 2 demonstrates, the break points on the long arm of chromosome 18 are indistinguishable in the 9/18 and the 4/18 translocations described above, yet in one case activity of the pep-A gene is lost, whereas in the other it is retained. Attention is therefore directed to the possible role of the translocated centromeric heterochromatin from chromosome 9.

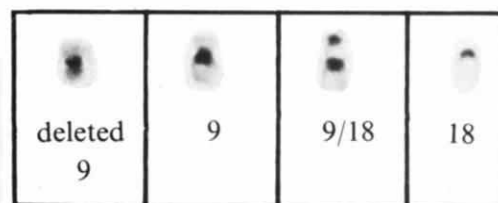


Fig. 2 Diagrammatic representation of the rearrangements involving chromosome 18 in the series of clones described in text. The break point on chromosome 18 involves the q23 region in both cases. The origin of the long arm material distal to the centromeric heterochromatin on the deleted chromosome 9 is uncertain.

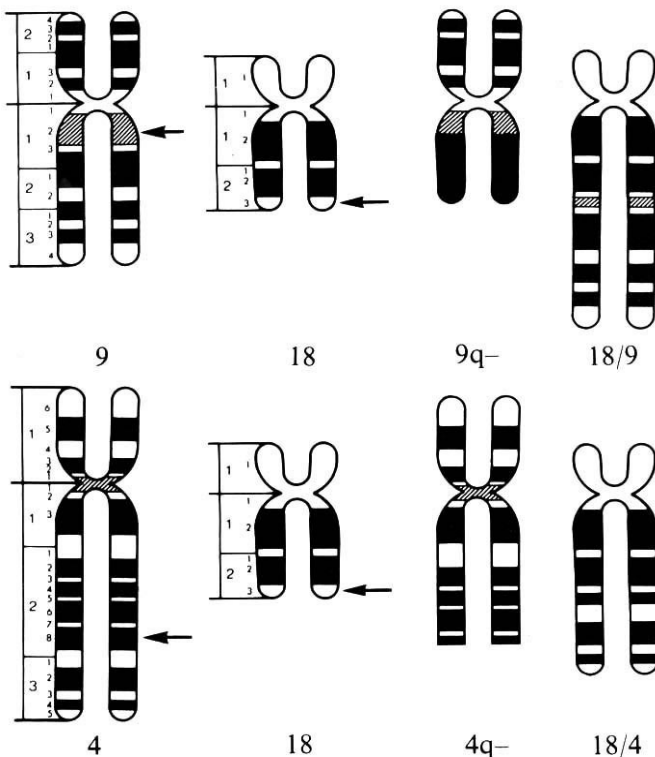


Fig. 3 C-banded preparation¹⁸ of cell from a pep-A-2 clone. The intercalary heterochromatin on the 18/9-derived chromosome is readily seen. There is some reduction in the amount of centromeric heterochromatin on the deleted chromosome 9.

In *Drosophila*, inactivation of a structural locus as a result of autosome-autosome translocation has been attributed to the suppressive effect of adjacent heterochromatin^{13,14}. (Similar gene suppression occurs when material from an autosome is translocated on to the inactive X chromosome in the mouse¹⁵.) It is usually varied in its incidence in individual cells, resulting in a variegated phenotype; further evidence that the locus in question is inactivated rather than deleted is provided if there is frequent reversion to give mosaicism or if reversions occur after recombination in which the affected locus and the adjacent heterochromatin become separated¹⁶.

One of the clones included in this study (J28) showed a very weak band in the pep-A-1 region, corresponding to approximately 10% of the normal pep-A-1 activity. On chromosome analysis every one of 30 cells examined had the 9/18 translocation shown in Fig. 1c. It is possible that this was an example of partial reactivation of the suppressed locus, although contamination with a few cells lacking the 9/18 translocation has not been completely ruled out. A second sample of this clone examined 1 month after recovery from storage in liquid nitrogen showed no detectable pep-A-1 activity.

Further studies are in progress with the aim of detecting revertants, with or without further chromosome change, in this and other pep-A-2 clones. If found, they will establish that a 'position effect' on human gene expression has been demonstrated for the first time.

Two other possible explanations for the present findings must be considered. First, since the distal fragment of the broken 18 is too small to be identified, it is possible that there has been reciprocal translocation, with retention of this fragment, in the 4/18 rearrangement but not in cells with the 9/18 translocation. Second, the break points of the 18 long arm may not be identical, being proximal to the pep-A-locus in one case but distal to it in the other. All three interpretations place the human pep-A structural locus, with some precision, in the q23 region of chromosome 18 (Fig. 2).

By virtue of their indefinite lifespan *in vitro* and their re-

lative genetic (including cytogenetic) stability, cultures of human lymphoblastoid cell lines offer considerable scope for further mapping of the human karyotype, particularly if data can be accumulated centrally from a number of laboratories over a prolonged period¹⁷.

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Received May 27; accepted August 19, 1975.

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Regulation of chromosome 21-directed anti-viral gene(s) as a consequence of age

WHEN human fibroblast cells are cultured *in vitro* they undergo about 50 cell population doublings at which time their growth rate begins to slow down and they finally die^{1,2}. For this reason, several investigators have used the senescence of human fibroblasts *in vitro* as a model system for experimental ageing research³⁻⁶. One explanation for human fibroblast senescence is that some cell functions are lost before cells reach their maximum division limit. We have described the existence of a complex regulatory gene function controlling the inducibility of chromosome 21-directed anti-viral gene(s) (AVG) in human fibroblasts⁷. Our conclusions were derived from experiments on human fibroblasts monosomic, disomic or trisomic for chromosome 21. We therefore used all three cell types in this study to test the preservation of this complex regulatory gene function in fibroblasts allowed to age *in vitro* and in fibroblasts derived from human donors of different ages. We found (1) that no differences exist in AVG expression in human fibroblasts allowed to age *in vitro* and (2) that the AVG in human fibroblasts derived from older human donors (64 yr) is easier to induce than it is in younger donors (0-29 yr).

Human fibroblasts trisomic (T-21), disomic (D-21) and monosomic (M-21) for chromosome 21 were cultured in 75-cm² Falcon flasks and maintained with Eagle's medium containing 10% heat inactivated foetal calf serum and 1% glutamine. The cultured cells were split at a 1:4 ratio every week for 8 months. Each split is designated as a passage number so, for example, cells split during week 15 are referred to as passage 15 cells.

Table 1 Inducibility of anti-viral gene(s) in human skin fibroblasts at different cell passage

Age of donor (yr)	Cell type	No. of cell population doublings	Units HuIF required to inhibit viral RNA synthesis by 50%
3	Monosomic 21	22	0.25
		32	0.24
		58*	0.20
		64*	0.24
		66†	0.22
1		18	0.22
		64*	0.20
5	Disomic 21	28	0.062, 0.059
		32	0.064
		40	0.059
		64	0.062
		76*	0.059
		78†	0.050
2	Trisomic 21	18	0.015
		22	0.016
		66*	0.015, 0.018
		68†	0.017

In those cases where more than one value is given, the additional values represent experiments done on different days.

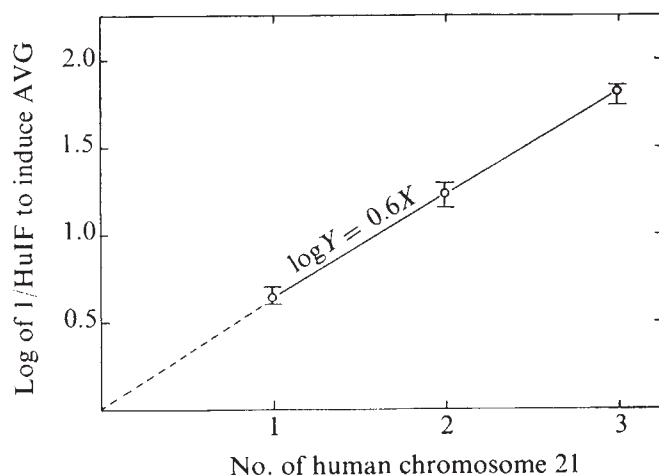
* Cell growth in phase III.

† No cell growth.

Each passage number is equivalent to two cell population doublings (passage 15 cells would have undergone 30 cell population doublings). The cells from one to two confluent flasks were frozen at each passage and stored in liquid nitrogen. The frozen cells were subsequently recultured and induced with human interferon. Concurrently the rates of cell growth were monitored at monthly intervals to determine the onset of cellular senescence. In all cases the rate of cell growth began to decrease by the time the cells had undergone 40–60 cell population doublings.

Normal diploid fibroblasts at different cell passage numbers were induced with various concentrations of human interferon. The inducibility of the AVG in these cells was assessed by the amount of human interferon required to inhibit viral RNA synthesis by 50% (ref. 7). Table 1 shows that the inducibility of the AVG remains unchanged in normal diploid fibroblasts in spite of ageing *in vitro*. Presumably this means that the regulatory gene function(s), known to control the inducibility of the AVG, also remains the same. The existence of a regulatory gene

Fig. 1 Gene dosage effect of chromosome 21 on the inducibility of the AVG in human fibroblasts monosomic, disomic and trisomic for chromosome 21. Each point represents the mean reciprocal concentration of human interferon required to induce the AVG in these cells and are derived from the values in Table 1.



function was previously demonstrated by the dosage effect of chromosome 21 on the amount of AVG product⁷. For this reason, the dosage effect of chromosome 21 on the inducibility of AVG was retested in early and late passage human fibroblasts containing differing numbers of chromosome 21. The results in Table 1 show that the dosage effect of chromosome 21 on the inducibility of the AVG remains unchanged in early and late passage M-21, D-21 and T-21 fibroblasts. These results were also plotted semi-logarithmically and a straight line represented by the equation, $\log Y = 0.6X$ (where Y denotes inducibility of the AVG and X denotes the number of chromosome 21 present) was obtained (Fig. 1). Previously, a dosage relationship expressed by the equation $\log Y = 0.61X$ was obtained⁷. The similarity in the two determinations suggests that there is no measurable loss of inducible AVG function and no significant change in the regulation of AVG expression in fibroblasts which have become senescent *in vitro*.

In the absence of measurable differences between early and late passage human fibroblasts we decided to compare the inducibility of the AVG in fibroblasts derived from human donors of different ages. In the following experiments, fibroblasts obtained from young humans (0–29 yr) are arbitrarily designated young fibroblasts and those from old humans (64 yr) are designated old fibroblasts. These fibroblasts were induced with various concentrations of human interferon and the inducibility of the AVG in these fibroblasts is presented in Table 2. The concentration of interferon required to induce the AVG in old fibroblasts is 2–3-fold lower than that required to induce the AVG in most of the young lines tested. One exception was a fibroblast line (GRC 35) derived from a 26-yr-old male which was shown to require about the same concentration of human interferon to induce the AVG as old fibroblasts. At

Table 2 Inducibility of the anti-viral gene(s) in normal skin fibroblasts derived from humans of different ages

Identification*	Age (yr)	Units of human interferon required to inhibit viral RNA synthesis by 50%
No. 141	5	0.062, 0.066, 0.063, 0.069, 0.070
GM 38	9	0.060
GM 181	10	0.059, 0.059
GM 72	11	0.060
GM 179	13	0.050, 0.050
GM 37	18	0.060, 0.052
GM 71	19	0.060, 0.062
GM 628	26	0.060
GRC 35	26	0.030, 0.040
GM 495	29	0.060
GM 288	64	0.020, 0.025, 0.028, 0.025
GRC 70	65	0.020, 0.028, 0.031
GRC 21	65	0.015, 0.015
GRC 48	73	0.015
GRC 26	75	0.030
GRC 34	80	0.030
GM 237	82	0.022, 0.040

* The GM series were obtained from Dr A. Greene of the cell repository at Camden, New Jersey. The remaining cell lines were kindly supplied by Dr E. Schneider of this laboratory. All of the cell lines were in early to early-middle passage numbers.

present such exceptions are difficult to explain. They may represent variant forms with enhanced sensitivity to the anti-viral action of interferon. The observed differences in the inducibility of the AVG in old and young fibroblasts suggest, however, that the structural gene element(s) which codes for the putative anti-viral protein or the receptor site for interferon are not as strongly repressed in old fibroblasts as in young fibroblasts. Others have also proposed similar mechanisms to explain the differential sensitivity of old and young chick embryo fibroblasts to chick interferon^{8,10}. This raises the question as to what cellular mechanisms are responsible for the differences in the different degrees of gene repression. One possibility may be that a nonspecific "relaxation" or "disorganisation" of the human genes occurs as a consequence of

age. Alternatively there may be selective pressures during the normal life span favouring the selection of cells based on their sensitivity to the anti-viral action of interferon.

It is apparent from this study that the regulatory gene function(s) controlling the inducibility of the AVG is not significantly changed in cells as a result of *in vitro* cell age.

We thank the Damon Runyon-Walter Winchell Cancer Fund for a grant. E.L.C. and N.L. are Damon Runyon-Walter Winchell fellows.

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Received March 27; accepted August 7, 1975.

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Mitochondrial suppressor of a nuclear gene in *Paramecium*

THE biogenesis of mitochondria depends on two sources of genetic information: one in the nucleus and the other within the mitochondria. An important question raised by this situation is how information from the two sources becomes integrated to form a functional organelle^{4,11}. Products coded by both mitochondrial and nuclear DNA have been shown to be assembled in the mitochondrial inner membrane to form complex enzymes such as ATPase, cytochrome oxidase, cytochrome *b* and mutations in either genome can affect enzyme activity^{2,6,7,14}. Since the activity of such enzymes depends on molecular interactions between their component subunits, it is conceivable that a defect in one subunit can be, at least partly, corrected by a mutation affecting another subunit. A nuclear mutation suppressing the effect of a mutated mitochondrial gene has been studied in *Neurospora*⁸. We report here the reverse situation.

The mutation was isolated in *Paramecium tetraurelia*, according to the new nomenclature¹³ and formerly *P. aurelia*, syngen 4. It is a mitochondrial mutation that partially suppresses the effects of the nuclear mutation *cl*₁. The properties of this mutation *cl*₁, already described¹⁰ are summarised first.

*cl*₁ is a monogenic nuclear recessive mutation and is characterised by (1) slow growth—a generation time of about 9 h at 27 °C instead of 6 h for wild type; (2) thermosensitivity—

most of the mutant cells die at 36 °C whereas the wild type grows as well as at 27 °C; (3) spectral deficiency in cytochrome oxidase—the absorption peak at 608 nm is about 10 times lower than in wild type; (4) incompatibility with mitochondria of wild-type origin (*M*⁺) and compatibility with those of mutant origin (*M*^{cl}): in contrast to the association between *cl*₁/*cl*₁ nucleus and *M*^{cl} mitochondria, the association *cl*₁/*cl*₁*M*⁺ results in a very reduced growth rate (generation time 15–20 h) and severely disorganised mitochondria. Although the basis (genetic, physiological or structural) of the difference between *M*⁺ and *M*^{cl} remains to be established^{3,10}, the *M*⁺ and *M*^{cl} states of mitochondria are stable and can be used as mitochondrial markers.

From the original *cl*₁ strain, a spontaneous fast growing revertant, *cl*₁-su, was isolated: its generation time at 27 °C is nearly that of wild type (7 h), although its amount of spectroscopically detectable cytochrome oxidase is not significantly different from that of the *cl*₁ mutant and its thermosensitivity is unchanged.

To establish the nature of this revertant, it was crossed with the wild type (Fig. 1A). The analysis depends on certain favourable features of the genetics of *P. tetraurelia*. The cytogenetic events of conjugation result in identical nuclear genomes in the F₁ clones derived from each ex-conjugant¹². Commonly, cytoplasm is not passed from mate to mate and so, the two clones arising from a pair of conjugants are like a pair of reciprocal crosses. In the cross performed here, therefore, one derives its cytoplasm from the wild type, the other from the revertant parent. The second generation is obtained from the F₁ clones by autogamy; if autogamy occurs in a clone heterozygous for a pair of nuclear alleles, 50% of homozygote cells for each allele are obtained in F₂ (ref. 12).

The results of the cross between the revertant (*cl*₁-su) and wild type (*cl*₁⁺), summarised in Table 1, reveal: (1) a 1:1 segregation in F₂ of the thermosensitivity property; (2) a difference between the two reciprocal crosses, ♀*cl*₁-su × ♂*cl*₁⁺ and ♀*cl*₁⁺ × ♂*cl*₁-su, as judged from the growth rate: a 1:1 segregation of slow-growing against normally growing cells appears only in the F₂ of the wild type cytoplasmic line. The phenotype of these slow growing cells clearly shows that the original *cl*₁ mutation was present and unchanged in the revertant: they have the typical generation time (15–20 h), thermosensitivity and mitochondrial alterations of the *cl*₁/*cl*₁*M*⁺ cells¹⁰. In the *cl*₁-su cytoplasmic line, the segregation of the alleles *cl*₁ and *cl*₁⁺ is only expressed by the segregation of the thermosensitivity character.

At least two hypotheses could account for these results: (1) the reversion could be due to a nuclear suppressor mutation. If so, it would have to be closely linked to *cl*₁ and would only suppress the slow growth of the *cl*₁/*cl*₁ cells when associated with *M*^{cl} mitochondria but not the interaction between gene *cl*₁ and *M*⁺ mitochondria. (2) The reversion could be due to a cytoplasmic suppressor (su). To test this second hypothesis the following experiment was carried out: the original *cl*₁/*cl*₁ strain, without suppressor, that is su⁺, was crossed with a *cl*₁⁺/*cl*₁⁺ strain derived from the *cl*₁-su cytoplasmic line of the previous cross (Fig. 1B), which was assumed to carry the

Table 1 Cross between the revertant (*cl*₁-su) and wild type (*cl*₁⁺)

Parents	Parental phenotype		F ₁ phenotype		Generation time (h)	F ₂ phenotype		No. of clones
	Generation time (h)	Thermo-resistance	Generation time (h)	Thermo-resistance		Thermo-resistance		
Revertant (cl ₁ -su)	7	—	6 – 7	+	7	+	58	
					7	—	62	
Wild type (cl ₁ ⁺)	6	+	6	+	6	+	73	
					15 – 20	—	76	

In the last column, the figures indicate the number of F₂ clones (from three different couples) studied. Average generation times at 27 °C are given. Thermoresistance or thermosensitivity at 36 °C is indicated by + or —. The cytoplasmic origin of each exconjugant F₁ clone was determined by its mating type, which is cytoplasmically inherited in this species¹².

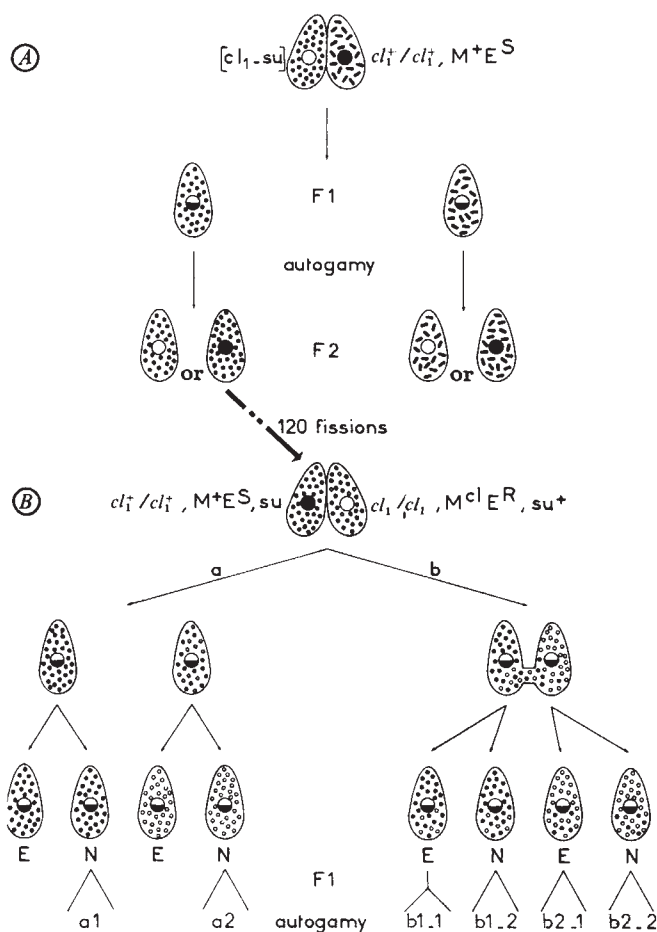


Fig. 1 Genetic analysis of the revertant. **A**, Cross between the revertant (cl₁-su) and wild type (cl₁⁺). ●, Cytoplasm of the revertant; ○, cytoplasm of wild type; ○, nucleus of the revertant; ●, nucleus of wild type; During conjugation, the reciprocal exchange of nuclei between the two parents yields two exconjugants (F₁) of identical heterozygote genotype ●, which differ only in their cytoplasm and therefore correspond to the two reciprocal crosses: ♀cl₁-su × ♂cl₁⁺ and ♀cl₁⁺ × ♂cl₁-su. Each exconjugant yields a clone and 20 generations later, autogamy can be induced in all cells of these F₁ clones. Autogamous cells then undergo a nuclear reorganisation which renders them homozygous for all their genes. If autogamy occurs in a clone heterozygous for a couple of nuclear alleles, a 1:1 ratio of the two homozygous genotypes is obtained in F₂ (ref. 12). **B**, Cross between a cl₁⁺/cl₁⁺ strain (●) and a cl₁/cl₁ strain (○) whose mitochondria carry a mutation for erythromycin resistance (M⁺E^R). This E^R mutation was selected from the original mutant strain which did not contained the suppressor (su⁺). From this cross, a pair (a) which had not undergone cytoplasmic exchange was studied as well as a pair (b) which had undergone a cytoplasmic bridge of 20 min. After the first post-conjugal fission in normal medium, one cell from each exconjugant was placed in erythromycin (150γ ml⁻¹) (E), the other was maintained in normal medium (N): as a result, clones b1-1 (after a lag of 2-4 d) and b2-1 became pure for M⁺E^R mitochondria, and clones b1-2 and b2-2 for M⁺E^S mitochondria. The F₂ analysis of these clones is given in Table 2.

postulated cytoplasmic suppressor, that is, su. Moreover, to test by this same cross whether the suppressor was mitochondrial or not, mitochondria from one parent (cl₁/cl₁) were marked by erythromycin resistance (M⁺E^R), those of the other (cl₁⁺/cl₁⁺) by erythromycin sensitivity (M⁺E^S). This is critical for the analysis, because it is possible in *Paramecium* to obtain matings in which cytoplasm (and mitochondria) of each mate pass to some extent into the other (Fig. 1B, b), so that each resulting clone has a mixed mitochondrial population (M⁺E^R + M⁺E^S). According to the medium (erythromycin or normal) in which these mixed clones are grown, one or the other type of mitochondria is selected^{1,9}. In this way, it is therefore possible to demonstrate whether the suppressor is associated with the mitochondria.

Two types of pairs were studied as explained in the legend of Fig. 1B; those without and those with cytoplasmic exchange between the two conjugants. Results obtained in F₂ for clones a1, a2, b1-1, b1-2, b2-1, b2-2 are given in Table 2. When no cytoplasmic exchange occurred between the two conjugants (pair a), 50% of cl₁/cl₁ cells (same generation time, 11 h, as the cl₁/cl₁M⁺E^R parent) and 50% of cl₁⁺/cl₁⁺ cells (generation time: 6 h) were obtained from the ex-cl₁ conjugant (a₂); all of these cells were E^R. In contrast, no segregation was apparent from the ex-cl₁⁺ conjugant (a₁): all the F₂ clones had a generation time of about 7 h and were E^S. This result means,

therefore, that the cytoplasm of the cl₁⁺ strain did carry the suppressor and maintained it over 120 cellular generations of association with a wild-type genotype (Fig. 1B). Furthermore, it can be seen that this suppressor also suppresses the incompatibility between gene cl₁ and mitochondria from the wild type, for the cl₁/cl₁M⁺su cells have a generation time of 7 h.

Information concerning the mitochondrial location of the suppressor can be derived from the study of the exconjugants which have exchanged cytoplasm (pair b). The presence of the suppressor in these exconjugant clones can be determined by the growth rate of the F₂ cl₁/cl₁ cells derived from them.

The mitochondrial composition of these clones (containing both M⁺E^R and M⁺E^S mitochondria after conjugation) will depend on the medium (E or N, Fig. 1B) in which they will be put, one fission after conjugation. In erythromycin, they will become pure for E^R mitochondria, in normal medium, pure for E^S ones^{1,9}. A very strict correlation between the presence or the absence of M⁺E^S mitochondria and the presence or the absence of the suppressor was observed (Table 2). When the F₂ progeny of a clone is E^S (b1-2), the generation time of the cl₁/cl₁ cells is identical to that of the cl₁⁺/cl₁⁺ ones and therefore the suppressor is present. When the F₂ progeny is E^R (b1-1 and b2-1), the generation time of the cl₁/cl₁ cells is 11 h: the suppressor is absent. The joint loss of E^S mitochondria and of the suppressor in clone b1 is particularly striking. Conversely, the joint gain of the E^S mitochondria and of the suppressor was observed in clone b2-2. While clones b1-1, b1-2, b2-1 had become pure for one mitochondrial type, clone b2-2 was still mixed (M⁺E^Rsu⁺, M⁺E^Ssu) when autogamy occurred: this was shown by the intermediate growth rate and resistance to erythromycin of the 13 cl₁/cl₁ cells when they were first tested (Table 2). Five fissions after autogamy, cells from these 13 clones were still mixed and could become pure for either E^R mitochondria and the su⁺ condition (generation time: 11 h)

Table 2 F₂ progenies of the cross cl₁⁺/cl₁⁺ M⁺E^Ssu × cl₁/cl₁ M⁺E^Rsu⁺ after growth in erythromycin (E) or normal medium (N) of the F₁ clones

F ₁ clones	a1 (N)	a2 (N)	b1-1 (E)	b1-2 (N)	b2-1 (E)	b2-2 (N)
Genotype	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺	cl ₁ /cl ₁	cl ₁ ⁺ /cl ₁ ⁺
Generation time (h)	7	11	6	11	6	8-9
Erythromycin resistance	—	+	+	—	+	±
No. of clones	85	39	34	14	15	13

The origin of the clones a1, a2, b1-1, b1-2, b2-1, b2-2 is given in Fig. 1B. The F₂ cells (cl₁/cl₁ and cl₁⁺/cl₁⁺) were tested for their resistance or sensitivity to erythromycin about three generations after autogamy and their growth rate was recorded. Average generation times at 27 °C are given. The erythromycin resistance or sensitivity is symbolised by + or —; ± indicates that the cells first were blocked in erythromycin and then progressively became resistant.

or for E^s mitochondria and the su condition (generation time: 7 h) according to the medium (E or N) in which they were grown.

A last point is worth consideration. This suppressor interacts not only with the product of the mutated gene *cl₁*, but also with the product of the wild-type allele, *cl₁⁺*: the *cl₁⁺/cl₁⁺su* cells have a slightly increased generation time (7 h instead of 6) and a strongly decreased cytochrome oxidase content (A. S., unpublished).

In conclusion, I have identified in the *cl₁-su* strain a suppressor which behaves genetically as a mitochondrial mutation, since its transmission is linked to the mitochondrial E^s marker. The mechanism of this suppression remains to be established. It is probably not a direct informational suppression because it is very unlikely that mRNA transcribed in the nucleus is translated by mitochondrial tRNAs⁵. It is more likely an indirect suppression by functional interaction between two modified products, the one nuclear, the other mitochondrial. The identification of the product coded by this new mitochondrial mutation should provide information on the informational content of the mitochondrial DNA and on the problem of the interactions between products coded by the nuclear and mitochondrial genetic systems.

I thank Dr J. Beisson for advice and discussions.

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Received June 19; accepted August 8, 1975.

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Spontaneous AKR lymphoma with T and B-cell characteristics

THE existence of surface immunoglobulin on thymus-derived cells (T cells) has been difficult to demonstrate and is still controversial¹⁻⁶. Therefore the presence of easily detectable surface immunoglobulin (sIg) is considered to be a reliable marker for lymphoid cells of bone marrow origin (B cells)⁷. To our knowledge no cell line has been reported to bear both easily detectable sIg (that is, by immunofluorescent staining) and T-cell markers, although two murine T-cell lines have been reported to bear small amounts of sIg detectable only by extremely sensitive methods^(2,8). We now report the existence of a murine lymphoma line with both T and B-cell characteristics by the criteria of easily detectable sIg, presence of Thy1.1 (θ AKR) antigen⁹, and mitogen responsiveness.

The tumour line, designated AkTB-1, originated as a spontaneous lymphoma in the lymph nodes and spleen of a 14-month-old AKR/J mouse which had been thymectomised at 1 month of age. Lymph node (LN) cells were initially passaged, and the line was subsequently maintained *in vivo* by serial intravenous passage of 10⁵ spleen cells into young AKR/J male hosts. For comparison, cells from age-matched normal AKR/J mice or from AKR/J mice with spontaneous thymomas were studied.

Thy1.1 antigen was detected by specific immunofluorescent staining using an indirect technique. Spleen or LN cells were incubated with anti-Thy1.1 antiserum prepared by hyperimmunisation of CBA/J mice with AKR/J thymocytes. The

Table 1 Thy1.1 antigen determinations

Donor	No. of experiments	% Positive cells ± s.e.	
		Spleen	LN
Normal 3 month AKR	6	38 ± 4	71 ± 3
AkTB-1 in passage*	9	94 ± 1	91 ± 1
AKR spontaneous thymoma	3	98 ± 1	98 ± 1
AKR lymphoma in passage†	8	96 ± 4	92 ± 2

*Experiments performed after day 12 of passage.

†Cell lines derived from spontaneous AKR thymomas passaged as spleen cells. One hundred cells were counted in each experiment.

cells were washed and then counterstained with fluorescein-conjugated goat anti-mouse γ₂ heavy chain antibody. The reactivity of this anti-Thy1.1 antiserum against thymus cells could be removed completely by absorption with AKR/J brain tissue, and less than 2% of AKR/J spleen cells react with anti-γ₂ antiserum.

Lymph nodes and spleens were removed from mice on various days after passage and examined for surface Thy1.1 antigen (Table 1). By day 12, more than 90% of LN and spleen cells were Thy1.1 positive. This pattern was maintained throughout the passage. Similar results were obtained with cells from AKR mice bearing a spontaneous thymoma or another passaged AKR lymphoma cell line derived from a spontaneous AKR/J thymoma.

sIg was determined by Dickler's method¹⁰. Cells were incubated with fluorescein-conjugated goat or rabbit antibody monospecific for α, μ, γ₁, and γ₂ heavy chains, as well as with a fluorescein-conjugated polyvalent anti-mouse immunoglobulin serum. The percentage of sIg positive cells was determined at various times after passage (Fig. 1). AkTB-1 spleen cells progressively acquired sIg of the IgM class; whereas fewer than 10% of cells were sIg positive on day 14, more than 90% were positive by day 22. At all times, fewer than 5% of cells could be stained for α, γ₁ or γ₂ heavy chains (data not shown).

Fig. 1 Percentage surface immunoglobulin positive cells in LN and spleen of AkTB-1 bearing animals. Open circles represent spleen cells; closed circles, LN cells. sIg represents staining for μ heavy chain on cell surfaces. Upper bracket shows range for normal spleen cells. Lower bracket shows range for normal LN cells.

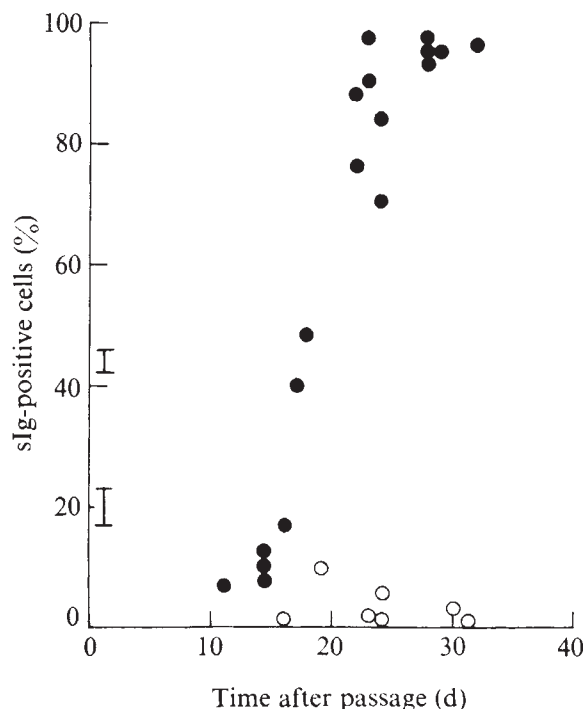


Table 2 Capping and regeneration of surface markers

	1 h after capping			24 h after capping		
	% Positive cells*	% Caps	% Rings†	% Positive cells	% Caps	% Rings
Thy1.1	100	75	25	100	0	100
sIg	74	89	11	77	16	84

*Expressed as percentage of total spleen cells.

†Expressed as percentage of positively stained cells; see text for explanation of terms. 2×10^7 spleen cells from an AkTB-1-bearing mouse late in passage were incubated with unlabelled anti-Thy1.1 antiserum for 30 min at 4 °C, washed and then incubated with unlabelled goat anti-mouse γ_2 heavy chain antibody for 1 h at 37 °C. Either at this point or after culture overnight, cells were examined for Thy1.1 antigens (see text). For detection of sIg regeneration, a single incubation at 37 °C for 1 h with unlabelled anti-mouse immunoglobulin was performed before overnight culture. Cells were stained according to method in text.

Thus almost all AkTB-1 spleen cells late in passage bear both IgM and Thy1.1 on their surface. Few LN cells from the same animals bore sIg even late in passage when lymph nodes were grossly and microscopically replaced with tumour. Neither spleen nor LN cells from three spontaneous or two other passaged AKR lymphomas had detectable (>2%) sIg of any class.

Since the coexistence of easily detectable T and B-cell markers on the same cell is unusual, we wished to test the possibility that either Thy1.1 or sIg was passively acquired. In one experiment (Table 2), Thy1.1 on the surface of AkTB-1 spleen cells was capped by incubating the cells first with unlabelled anti-Thy1.1 serum, then with unlabelled anti-mouse γ_2 heavy chain at 37 °C¹¹. Cells stained immediately after these incubations were 100% positive for Thy1.1; in 75% the antigen was present only in the form of caps, while 25% still had a circumferential (ring) distribution of Thy1.1. After overnight incubation, cells reacted with Thy1.1 antisera and counter-stained with fluorescein-conjugated anti- γ_2 antibody all exhibited a fluorescent ring pattern. Staining with only anti- γ_2 showed 92% of cells were still capped, indicating that the Thy1.1 caps formed a day earlier had neither shed nor redistributed themselves over the cell surface. These results suggest that AkTB-1 spleen cells can generate Thy1.1 antigen.

Similar results were obtained for capping and regeneration of surface immunoglobulin (IgM) on AkTB-1 spleen cells (Table

2). In addition, preliminary studies indicate that these cells synthesise IgM *in vitro*.

Mitogen-stimulated DNA synthesis by AkTB-1 cells was determined using a microculture method. Responsiveness to phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) at early, middle and late points in a typical passage are shown in Table 3. At day 7 of passage, when lymphoid organs were not yet enlarged and the frequency of Thy1.1 positive spleen cells was only slightly increased, mitogen responsiveness of AkTB-1 spleen cells was comparable with that of normal cells. By day 14, when lymphoid organs were enlarged 10-fold and spleen cells were 94% Thy1.1 positive and 33% sIg positive, spontaneous DNA synthesis was elevated, but was depressed by PHA and LPS. Late in passage when AkTB-1 spleen cells were 95% Thy1.1 and 83% sIg positive, they exhibited a high spontaneous level of DNA synthesis which was depressed by PHA but markedly stimulated by LPS. Mitogen responsiveness of AkTB-1 LN cells (Table 3) was not remarkably different from that of normal AKR cells. These results further suggest the dual nature of the spleen cells, since LPS responsiveness is considered to be characteristic of B cells¹², whereas suppression of DNA synthesis in response to PHA has been reported as a characteristic of some murine T-cell lymphoma lines^{13,14}.

AKR lymphoma usually arises in the thymus, presumably as a result of viral transformation of a susceptible cell population, and can be prevented by thymectomy¹⁵. The

Table 3 Mitogen-stimulated ³H-thymidine incorporation by AkTB-1 and normal lymphocytes

	AkTB-1		Normal	
	Spleen	LN	Spleen	LN
Day 7				
Thy1.1 (%)	64	72	32	—
sIg (%)	49	16	48	—
Yield*	1×10^8	4×10^7	8×10^7	—
Medium†	829 ± 69	120 ± 11	$1,051 \pm 316$	318 ± 77
PHA	$16,651 \pm 1,251$	194 ± 6	$23,518 \pm 1,338$	$19,152 \pm 1,317$
LPS	$9,615 \pm 1,403$	128 ± 88	$15,236 \pm 661$	324 ± 74
Day 14				
Thy1.1	94	94	25	70
sIg	33	18	44	20
Yield	1×10^9	5×10^8	1×10^8	3×10^7
Medium	$29,564 \pm 887$	$2,405 \pm 382$	$1,872 \pm 329$	$2,537 \pm 988$
PHA	$10,253 \pm 408$	$5,708 \pm 324$	$9,319 \pm 1,880$	$5,231 \pm 1,229$
LPS	$8,055 \pm 978$	$1,962 \pm 848$	$5,932 \pm 853$	$2,318 \pm 495$
Day 21				
Thy1.1	95	95	32	72
sIg	83	5	38	18
Yield	1×10^9	5×10^8	9×10^7	6×10^7
Medium	$35,493 \pm 1,791$	366 ± 53	501 ± 27	305 ± 25
PHA	$17,554 \pm 1,495$	$2,451 \pm 5$	$3,994 \pm 135$	$2,177 \pm 205$
LPS	$83,733 \pm 1,508$	$1,148 \pm 175$	$5,273 \pm 250$	352 ± 5

*Yield indicates total number of nucleated cells in spleen or lymph nodes (inguinal, axillary and mesenteric).

†Data are mean c.p.m. $\pm$ s.e. of triplicate cultures at the mitogen concentration resulting in optimal stimulation (PHA, $1.6 \mu\text{g ml}^{-1}$; LPS, $100 \mu\text{g ml}^{-1}$).

Cells were suspended at a concentration of 10^7 cells per ml in RPMI 1640—10% FCS with 2 mM fresh glutamine and antibiotics. 0.1 ml of cells was incubated in microtitre plates with an equal volume of medium or doubling dilutions of PHA or LPS. The cultures were incubated at 37 °C in 5% CO₂ in air for 18 h, then pulsed with $1.0 \mu\text{Ci } ^3\text{H}$ -thymidine for a further 6 h before collection.

origin of the AkTB-1 tumour line as a spontaneous lymphoma in a thymectomised mouse suggests that the absence of the thymus was important in the generation of the unusual double-marker characteristics of these cells. This hypothesis is supported by the recent appearance in our laboratory of a second sIg positive, Thy1.1 positive spontaneous tumour originating in another thymectomised AKR/J mouse. It may be that the absence of susceptible thymus cells allows the subsequent transformation of a normal double-marker cell population. Such a subpopulation has been described in man¹⁶⁻¹⁸. Alternatively the thymus may modulate neoplastic as well as normal cell differentiation, so that in its absence transformed cells develop atypical characteristics.

Regardless of the origin of these cells, the progressive acquisition of B-cell characteristics as well as their differential expression in spleen and lymph node suggest that these neoplastic cells undergo differentiation *in vivo* and that the lymphoid tissue microenvironment has a role in the control of this differentiation process.

We thank Dr Richard Asofsky and Ms Pamela Sharp for help in providing antisera and studying immunoglobulin synthesis and markers. We also thank Drs William Terry and Howard Dickler for advice and criticism.

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Received June 18; accepted July 30, 1975.

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Precommitment of normal mouse peritoneal cells by erythrocyte antigens in relation to auto-antibody production

We have reported that peritoneal cells (PCs) from unstimulated mice can, after 2 h of incubation in the appropriate medium, start to secrete anti-sheep erythrocyte (SRBC) antibodies^{1,2}. We extended this observation by showing that PCs cultured in standard conditions for 4-6 d in the absence of SRBC, can form numerous haemolytic plaques, demonstrable by the ordinary techniques of local haemolysis (in Agarose, in liquid layer or in cellulose gum³). The immunological nature of this plaque-forming activity was established by the following criteria: complement dependency, inhibition by anti-mouse IgM serum and immunological specificity. Thus, we concluded^{3,4} that PCs of normal adult mice had been stimulated previously by an unknown immunogen sharing common or cross-reacting determinants with SRBC, and that tissue culture conditions would derepress the built-in capacity of these cells to produce antibodies. Treatment of autologous erythrocytes by the proteolytic enzyme bromelain revealed that normal mouse spleen cells

regularly produce antibodies against their own erythrocytes^{5,6}. This led us to investigate the behaviour of normal PCs towards SRBC or mouse red blood cells (MRBCs) treated by bromelain (BrMRBCs). We report here that mouse PCs in culture develop a plaque-forming activity against isologous BrMRBC as well as against SRBC, and we show that these two types of erythrocytes share some common antigen.

PCs from non-immunised 22-28-week-old CBA/J or NZB mice were cultured by Mishell and Dutton's technique⁷ at a concentration of 4×10^6 ml⁻¹ in RPMI 1640 supplemented with 10% foetal calf serum. Cell viability was measured by Trypan blue exclusion. The plaque-forming activity of lymphoid cells was measured by Cunningham's liquid layer technique⁸ or the carboxymethyl cellulose technique (CMC)^{4,9}. SRBCs or MRBCs were treated with bromelain as described by Cunningham⁵. For mixed plating, an equal number (5×10^8 ml⁻¹) of BrMRBCs plus SRBCs or of BrMRBCs plus BrSRBCs was used. A mixture of equal volumes of fresh rabbit and guinea pig sera was used as a source of complement, the final concentration being 10%.

PCs from normal mice produced no plaques of haemolysis against MRBC in the liquid layer technique, and only an insignificant background was detected by day 4 of culture with the CMC technique (Table 1). On the other hand, as already described³, increasing number of plaque-forming cells (PFCs) developed against SRBCs during culture. The highly sensitive CMC technique¹⁰ provided many more plaques than the liquid technique.

If mouse erythrocytes were treated with bromelain their antigenic properties were modified so that they became sensitive to antibodies secreted by mouse PCs (Table 1). In most cases treatment of SRBCs by bromelain also led to an increase of the PFCs expressed by PCs in culture.

The behaviour of spleen cells from normal mice cultured in the absence of SRBCs was similar to that of PCs: although almost no PFCs against MRBCs and only a few background plaques against SRBCs could be detected, treatment of these erythrocytes with bromelain resulted in the appearance of a substantial number of PFCs in the spleen cell population (Table 2).

The immunological nature of the plaque-forming activity against BrMRBCs and BrSRBCs was demonstrated by the fact that no plaques appeared in the absence of complement and that 95% of PFC were inhibited by a specific anti-mouse IgM serum, added to the liquid medium or CMC at a final concentration of 1%. The system displayed immunological specificity inasmuch as horse red blood cells (HRBCs) or bromelain-treated HRBCs never gave any significant number of plaques with normal peritoneal or spleen cells (SCs). Furthermore, ghosts or SRBCs or BrMRBCs, obtained as described by Dodge *et al.*¹¹, inhibited plaques against SRBCs and BrMRBCs whereas ghosts of HRBCs did not.

As both SRBCs and BrMRBCs could be lysed by cultured peritoneal and spleen cells it was tempting to speculate that the cells share common or cross-reacting determinants. To check this hypothesis, both sorts of cells were plated with a mixture of both types of erythrocyte, and the morphology of plaques of haemolysis was examined¹². Three types of plaque were observed: (1) clear plaques, (2) 'sombrosos', where the centre was clear and the periphery cloudy and (3) incomplete or cloudy plaques. Clear plaques and sombrosos were judged as indicating cross-reactivity between antigenic determinants on the surface of both types of erythrocyte present in the gel, whereas cloudy plaques were thought to be formed in the absence of such cross reactivity. As Table 3 shows, SRBCs and BrMRBCs cross reacted slightly (10%) while BrSRBC and BrMRBC cross reacted to a large extent (40%).

These results lead us to interpret the spontaneous plaque-forming activity of PCs, and at a lower level of spleen cells, as a manifestation of an autoimmune process. As Cunningham proposed¹³, young mice would be sensitised to antigenic determinants present on their own erythrocytes but hidden in the

Table 1 Development of spontaneous plaque-forming cells by CBA mouse peritoneal cells in culture

Days in culture	Plaque assay system	MRBC	Plaque-forming activity* of PCs plated on different types of erythrocytes	BrMRBC†	SRBC	BrSRBC†	HRBC	BrHRBC†
0‡	Liquid§	0	2±4	0	0	0	0	0
	CMC	0	3	24±5	79±15	0	0	0
4	Liquid	0	3,963±425	ND	ND	1±4	4±5	4±5
	CMC	2±4	29,592±3,899	7,169±1,472	6,918±762	5±2	4±5	4±5
	Liquid	0	5,700¶	1,313±259	3,484±951	ND	ND	ND
6	CMC	0	31,510±3,950	8,379±2,339	27,490±3,180	ND	ND	ND

* Plaques per million viable recovered cells ± s.e.

† Bromelain-treated mouse (M), sheep (S) or horse (H) red blood cells.

‡ Peritoneal cells were collected, washed and placed directly into plaque assay medium without previous culturing.

§ Carboxymethyl cellulose gel; readings after 2 h at 37 °C.

|| Readings after 1 h at 37 °C.

¶ Approximate number; at the dilution used the number of plaques per slide was too high to be counted accurately.

Table 2 Development of spontaneous PFCs by CBA mouse spleen cells in culture

Days in culture	Plaque-forming activity† of SCs plated on different types of erythrocytes	MRBC	BrMRBC†	SRBC	BrSRBC†
0‡	3±5	176±53	11±2	340±88	
1	1±4	233±35	10±10	400±60	
2	7±8	1,273±191	5±8	268±85	
4	0	823±179	51±26	145±32	

* Plaques per million viable recovered cells ± s.e., counted after 1 h at 37 °C; liquid medium technique.

† Bromelain-treated mouse (M) or sheep (S) red blood cells.

‡ Spleen cells were collected, washed and placed directly into plaque assay medium without previous culturing.

Table 3 Formation of clear plaques, sombreros and incomplete plaques by peritoneal cells in culture when plated with a mixture of SRBC and MRBC

Indicator cells	Activity of the peritoneal cells (PPM†)			
	Clear plaques	Sombreros	Incomplete	% double plaques§
SRBC (1)	2,233±496	0	0	
(Br)SRBC* (2)	5,300±662	0	0	
MRBC (3)	0	0	0	
(Br)MRBC* (4)	11,766±3,636	0	0	
MP‡ (1)+(3)	0	0	1,360±296	0
MP‡ (1)+(4)	67±103	766±585	7,533±1,998	9.4±5.2
MP‡ (2)+(4)	1,640±712	2,400±316	6,080±1,180	40±7.9

NZB peritoneal cells were cultured for 6 d (CBA mouse PC gave comparable results). 'C' was a mixture of equal parts of guinea pig serum and rabbit serum checked for absence of haemolytic activity against the erythrocytes used.

* Bromelain-treated sheep (S) or mouse (M) red blood cells.

† Plaques per million recovered viable cells ± s.e.; readings were performed after 1 h at 37 °C, liquid medium technique.

‡ MP, mixed plating; red cells were mixed in equal proportions as indicated.

§ Number of clear and sombrero plaques as percentage of the total plaque number.

membrane. If these epitopes, unravelled on MRBC by bromelain, are also present in an exposed form on SRBCs, auto-antibody secreted by peritoneal and spleen cells would attach spontaneously on SRBCs.

A repressive mechanism seems to restrict the number of PFCs *in vivo* to a low level¹³. Our results show that, in culture conditions, their number increases considerably. Thus, the immune behaviour of PCs becomes a phenomenon of general biological importance, and perhaps an index of a spontaneous autoimmune process. Our findings underline the need for a reassessment of the concept of 'primary response' of mice to SRBCs.

We thank Danielle Arnaud for technical assistance and the CNRS and INSERM for support.

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Received June; accepted August 13, 1975.

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Noradrenaline induces morphological alterations in nucleated and enucleated rat C6 glioma cells

GILMAN and Nirenberg¹ have reported that catecholamines induce a striking increase in intracellular cyclic AMP concentration in cultures of rat and human glioma cells. They concluded that this activation of the adenylate cyclase was mediated through the β -adrenergic receptor, since the effect was inhibited by the β -receptor blockers sotalol and dichloroisoproterenol. Another report² showed that cultured human glioma cells treated with cyclic AMP derivatives resumed the typical morphology of the normal counterparts, that is, having multiple processes extending from a compact cell body. I have therefore compared the effects of noradrenaline, a β -adrenergic stimulator, on the morphology and cyclic AMP level in rat C6 glioma cells.

I have also investigated whether the variations in cell shape require nuclear activity. The results demonstrate that the morphological alteration occurs in normal cells as well as in cells enucleated by cytochalasin B. Moreover, the effect of noradrenaline on cyclic AMP concentration and cellular morphology is transient.

In the absence of noradrenaline (Sigma) the nucleates and the enucleates have an irregular flattened shape (Fig. 1a and c, respectively). Treatment of the C6 cells and the enucleates with the drug produced a marked change in morphology; the cells and the enucleates assumed a spindle-like morphology (Fig. 1b and d, respectively). The cytoplasm retracted to form a compact cell body with multiple processes. Occasionally the treated nucleates as well as enucleates had bipolar processes. Now and then the processes showed beadings and bifurcations. Routinely, I used 10^{-5} M noradrenaline; concentrations as low as 10^{-7} M are sufficient to induce the morphological change, whereas 10^{-6} M noradrenaline has no effect.

The effects of noradrenaline on the morphology of the nucleates and the enucleates was inhibited by the β -receptor blocking agent DL-propranolol (Sigma, at 10^{-5} M), but not by phentolamine (Ciba-Geigy, 10^{-6} - 10^{-4} M), a specific α -receptor blocker.

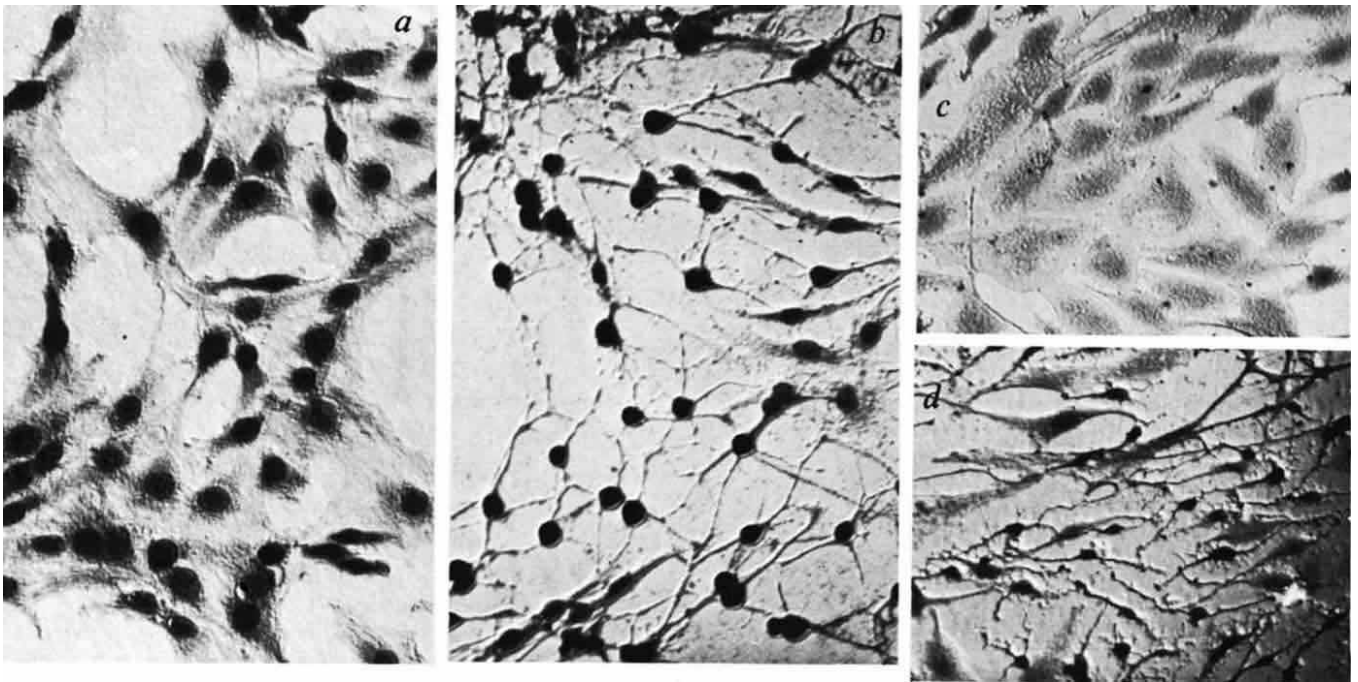


Fig. 1 Demonstration of the morphological change in nucleated and enucleated rat C6 glioma cells caused by noradrenaline. The C6 rat glioma cells used throughout the experiment were constructed by Benda *et al.*³ and were obtained from Flow Laboratories. The cells were routinely maintained in Ham's F10 medium (Flow Laboratories, contains 0.25 mM Ca^{2+}) supplemented with 15% horse serum and 2.5% foetal bovine serum in a humidified 5% CO_2 incubator at 37 °C. In the actual experiments, the cells were allowed to recover from trypsinisation for at least 48 h in fresh medium. For the enuclea-

tion of C6 cells, the protocol described by Prescott *et al.*⁴ was followed, which in the case of C6 cells yielded more than 90% enucleation. The enucleates were then placed back in the original medium at 37 °C for 30 min to allow them to assume a normal appearance before starting the experiments. Nucleates (a) and enucleates (c) in the absence of noradrenaline and in the presence of 10^{-5} M of noradrenaline for 2 h (b and d, respectively). The cells were fixed in 100% methanol and stained with Giemsa. The micrographs were taken with Zeiss interference contrast optics. Magnification $\times 224$.

The morphological change in C6 cells, nucleates and enucleates, stimulated by noradrenaline, could be inhibited by colchicine (Serva, 1 mg ml^{-1}), added together with noradrenaline. Cells already induced by noradrenaline assumed their control appearance promptly after addition of colchicine. These observations suggest that polymerisation of the microtubules is involved in the formation of processes in C6 cells.

The morphological change in C6 cells could be observed 30 min after addition of noradrenaline (Fig. 2). At this time about 20% of the cells showed the characteristic processes. The fraction of morphologically altered cells then increased approximately linearly with time. At 4 h more than 90% of all cells

assumed the new morphology. From this time on, more and more cells looked normal once again; and 8 h after the application of noradrenaline all cells were morphologically indistinguishable from untreated cells.

In separate experiments I measured the cyclic AMP level in the cells during their morphological modulation (Fig. 2). Untreated cells had a basal level of 10 ± 4 pmol per mg protein, which is similar to that found in many tissues, including brain. But after treatment with noradrenaline the cyclic AMP concentrations increased strikingly, more than 100-fold, in agreement with previous observations by Gilman and Nirenberg¹. After 30 min the cyclic nucleotide concentration began to decrease and at 4 h it reached the 2–3-fold basal levels. The cyclic AMP level in the cells dropped to the basal value at 8 h.

The data of Fig. 2 show that most of the cells are morphologically altered when the cyclic AMP level has already dropped to almost basal levels (at 4 h). This time course indicates that both effects of noradrenaline are transient. Moreover, I have

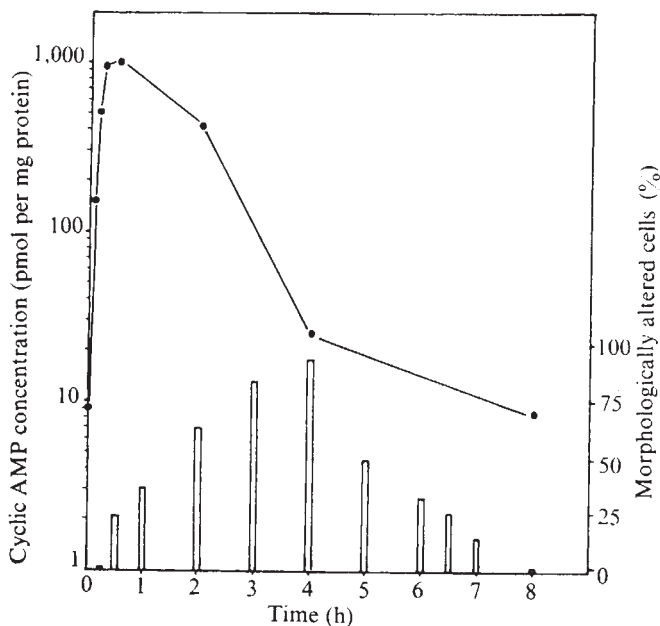


Fig. 2 Time course of morphological alteration (bars) and cyclic AMP concentration (black circles) in nucleated C6 cells induced by 10^{-5} M noradrenaline. Parallel cultures of C6 cells grown in 6 cm dishes (Greiner) were inspected for morphological alteration. The intracellular cyclic AMP concentration was then determined in the parallel culture. Before the experiment was started, C6 cells were incubated in medium for 48 h after replating. The cells were fixed with 100% methanol and stained with Giemsa. A total of 500 cells per dish were examined for the type of morphological alterations shown in Fig. 1. For determination of cyclic AMP the medium was quickly removed by aspiration and the cells were washed twice with PBS at 37 °C and fixed with 2 ml of ice-cold 5% trichloroacetic acid. The cells were then scraped off the dish. The protein debris was removed by centrifugation and the protein concentration was determined by the method of Lowry, using bovine serum albumin (Boehringer Mannheim) as standard. The supernatant was extracted five times with ether, which was presaturated with HCl-acidified water. The samples were then freeze-dried. The dry residue was suspended in 0.2 ml of 0.2 M sodium acetate buffer, pH 4. The cyclic AMP concentration was determined according to Gilman⁵.

found that the cells did not respond to a second treatment with noradrenaline for at least 60 h. After this refractory period, noradrenaline once again induced the characteristic modulation of cyclic AMP and morphology. Addition of dibutyryl cyclic AMP (Boehringer Mannheim, 1 mM) plus theophylline (Sigma, 0.1 mM) also induced the morphological change in C6 cells.

Taken together, these results suggest that noradrenaline stimulates by way of the β -adrenergic receptor, the adenylate cyclase of C6 cells, which then acquire a morphology similar to that of the astrocytes in brain tissues. Both changes are transient and are followed by a long refractory period. There are some reports^{6,7,8} in which cyclic AMP has been implicated in tubulin polymerisation in cells, but not in a cell-free system⁹. The morphological alteration in C6 cells is prevented by colchicine, again indicating the participation of microtubules. This was directly shown for C6 cells after stimulation by noradrenaline, (Weber, personal communication) using the immunofluorescence technique for specific visualisation of cytoplasmic microtubules¹⁰.

My experiments and other reports^{7,11,12} demonstrate that the information necessary for normal cell shape and morphological change is preserved in the cytoplasm of cells enucleated by cytochalasin B. The system described here may be useful for seeking a possible causal relationship between cyclic AMP and polymerisation of microtubules.

I thank Drs R. Knippers and K. Weber for their interest in this work, Dr H. Sauer for reading the manuscript and Mr B. Schlegel for assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

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Received July 1; accepted July 29, 1975.

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Genetically determined defect of Schwann cell basement membrane in dystrophic mouse

FOR more than a century there has been controversy as to whether Schwann cells are able to synthesise collagen and the collagen-related basement membrane^{1,2}. Recent evidence provides more direct proof that Schwann cells are indeed equipped to synthesise collagen and probably also a basement membrane in the same way as other cells of epithelial and neuroectodermal origin^{3–5}. Observations in various pathological situations, including regeneration after crush injury^{6,7} and in peripheral nerve ischaemia⁸ indicate that axon regrowth and Schwann cell migration occur along scaffolding consisting of basement membranes and endoneurial collagen fibres. The role of the basement membrane and the endoneurial collagen in normal myelination has, however, remained largely unknown. It has been generally accepted that these extracellular elements provide not only mechanical support

to peripheral nerves, but also that they are partly responsible for the relative lack of developmental faults in peripheral myelination⁹. A recent report¹⁰ suggests that the acquisition of a basement membrane by a Schwann cell occurs at the time that the cell establishes a permanent relationship with an axon. It is not known, however, whether the association of the axon with the Schwann cell induces the latter to synthesise basement membrane, or whether the Schwann cell only establishes a relationship with an axon once the Schwann cell has acquired a basement membrane.

There are at least three ways of investigating this problem: (1) by making serial observations during embryogenesis; (2) by studying the effects of selective damage to basement membrane and collagenous elements on myelination; or (3) by studying the mutant dystrophic mouse, in which areas of apparent early arrest of Schwann cell development and myelinogenesis are known to occur, particularly in the proximal peripheral nervous system^{11–13}. Our observations on the dystrophic mouse indicate that a genetically determined partial deficiency of basement membrane is present in the Schwann cells of the peripheral nervous system, and suggest that the Schwann cells develop basement membrane before establishing a definitive relationship with the axons.

Dystrophic (*dy/dy*) mice of 1, 2, 3 and 5 months of age were studied. Phenotypically normal littermates of the dystrophic animals (+/?) and C57BL mice were used as controls for each age group. They were prepared for electron microscopy as reported previously¹¹. Segments of the sciatic nerve 4–5 mm above the sciatic notch were studied at all time points, and at 3 months of age the common peroneal nerve was also examined.

A consistent finding in all dystrophic mice was a patchy deficiency of the basement membrane of Schwann cells of fibres which were myelinated (Fig. 1). The basement membrane was completely absent in amyelinated zones. In longitudinal sections, myelinated fibres entering an area of amyelination lost not only their Schwann cell covering and myelin sheath, but also the associated patchy basement membrane (Fig. 2). Some of the myelinated fibres had abnormally long bare nodal segments, without the usual basement membrane and Schwann cell pseudopodial covering. The abnormally long bare nodes are probably responsible for the 25% reduction in the sciatic nerve conduction velocity found by Huizar *et al.*¹⁴. Skin basal cells, skeletal muscle fibres, and endoneurial blood vessels showed continuous basement membrane of normal thickness. The basement membrane of the normal littermates (+/?) was normal, and identical to that in the normal C57BL mice. The defect of basement membrane was thus inherited in an autosomal recessive manner in these dystrophic mice, and presumably the gene responsible for the dystrophy is also responsible for the basement membrane defect.

Peripheral nerve development in the normal foetus involves migration of Schwann cells around bundles of axons, the progressive division of these bundles into smaller and smaller groups by proliferating Schwann cells, and the eventual segregation and enwrapping of axons by Schwann cells^{15–17}. The exact final relationship depends on whether the axon is to become a myelinated or an unmyelinated fibre. The observations of Webster and colleagues¹⁰ on tadpole nerve indicate that migrating Schwann cells lack basement membrane, which only forms when the Schwann cell establishes a permanent relationship with an axon. In mammalian foetal nerve the Schwann cells taking part in the segregation of axon bundles already have basement membranes^{18,19}. Our studies of the mutant dystrophic mouse show that Schwann cell basement membrane is patchily deficient throughout the peripheral nervous system, and that uncommitted (presumably Schwann) cells around amyelinated nerve fibre bundles in the nerve roots have a similar, and sometimes more marked, deficiency of basement membranes. This basement membrane deficiency is probably the result of impaired Schwann cell function, rather than of an inadequate axonal signal to the Schwann cell. The basement membrane deficiency in the

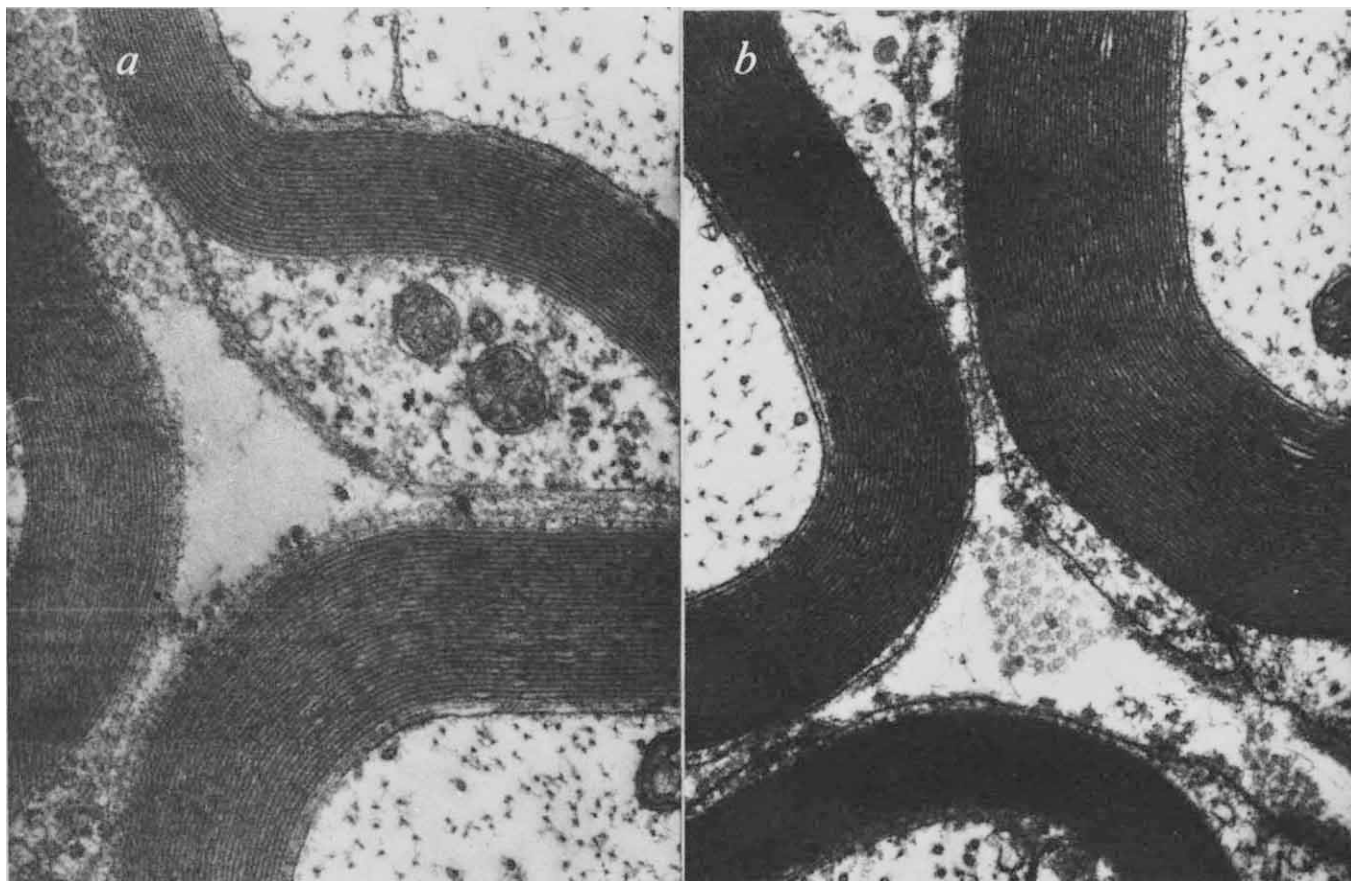


Fig. 1 Two-month-old mouse upper sciatic nerve showing portions of 3 myelinated nerve fibres. *a*, Normal C57BL; *b*, *dy/dy* mouse. In *a* the basement membrane over the Schwann cells is complete, and the minimum separation of 2 Schwann cell plasma membranes is 78 nm. In *b* the dystrophic basement membrane is patchy and incomplete, which allows the plasma membranes of two Schwann cells to approach to within 55 nm in this figure (middle). In some instances, dystrophic Schwann cells plasma membrane approached close enough to form an intraperiod line. In *b* there is apparently abnormally wide separation of the leaflets of the Schwann cell plasma membrane. $\times 45,000$.

dystrophic mouse may therefore simply represent a deficiency of Schwann cell function; there is already suggestive evidence of other Schwann cell abnormalities including an oligodendroglial-like arrangement of some Schwann cells (E. J., R. M.

and W. G. B., unpublished), and of aberrant axonal enwrapment and myelination¹¹.

It is possible to advance an hypothesis relating the peripheral nervous system abnormalities to the basement membrane deficiency. Let us suppose that synthesis of the basement membrane were necessary to terminate Schwann cell migration, and to establish a permanent relationship between the Schwann cell and an axon. The decreased ability to synthesise basement membrane characteristics of the dystrophic mutant Schwann cell would result in a greater tendency for Schwann cells to migrate further from the neural crest. Fewer Schwann cells would therefore come to rest in the spinal roots, and those which did settle, both in the roots and more peripheral parts of the nervous system, would show a decreased capacity for axonal enwrapment and myelinogenesis.

There are, however, still some features of the dystrophic mouse peripheral nervous system which are not fully explained by this hypothesis. These include the exact distribution of the neural abnormality, the presence of uncommitted Schwann cells around amyelinated axons, and the aberrant myelination in the spinal roots^{11,12}.

To the best of our knowledge this is the first reported incidence of a genetically determined deficiency of basement membrane production of Schwann cells, though abnormalities of the basement membrane have been incriminated in other diseases. In Alport's syndrome (hereditary nephropathy with nerve deafness), the basement membrane of renal glomeruli shows focal thickening, thinning and splitting, and this abnormality is genetically determined²⁰. Deficiency of epithelial basement membrane is believed to be of importance in the invasiveness of carcinoma^{21,23}, and has also been reported in dermatitis herpetiformis²⁴. The Schwann cell of the mutant dystrophic mouse may therefore prove to be an equally useful

Fig. 2 Two-month-old *dy/dy* mouse upper sciatic nerve showing an axon in longitudinal section. To the left is a paranodal termination of the Schwann cell cytoplasm and its myelin sheath. In the middle and right, the axon is naked with neither Schwann cell nor basement membrane covering. $\times 4,500$.



tool to those interested in basement membranes, peripheral nerve development and oncology.

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Mosaicism in organisation of concanavalin A receptors on surface membrane of mouse egg

PLANT lectins have proved to be useful probes of the surface properties of a variety of cells and membranes, providing data on the relative mobility of intramembranous macromolecules in different conditions¹ and on the organisation of oligosaccharides on the cell surface. We report here the use of fluorescent concanavalin A (con A) as a probe to analyse the surface properties of the unfertilised and fertilised mouse egg. Similar experiments were carried out independently at both Cambridge and Birmingham and the results are combined.

The fluorescent staining pattern (for details, see Fig. 1) of unfertilised eggs was not uniform. An area of weak or absent staining, designated the 'negative area', was consistently detected, occupied approximately one-fifth of the egg's surface and was associated in some but not all eggs with a slight distension of the cytoplasmic membrane (Figs 1a and 2). Cytological investigation and vital staining with acridine orange revealed that the negative area was consistently associated with the underlying second metaphase spindle. After incubation at 20 or 37 °C the negative area was separated from positively stained membrane by a sharply defined boundary line (Fig. 1b), but the boundary appeared more diffuse after incubation at 4 °C. The higher temperatures also affected the pattern of staining within the positive area converting it from a dullish even fluorescent staining to a bright patchy staining pattern at concentrations of con A in excess of 50 µg ml⁻¹ (Fig. 1c).

Various drugs were examined for their effects on the staining pattern. Preincubation in neuraminidase (50 or 100 IU ml⁻¹, Behringwerke) did not affect the staining pattern. Sodium azide at 0.5 × 10⁻² M tended to exaggerate the punctate staining of the positive area and caused the area

of negative stain to contract in size (Fig. 1c) and in many eggs to disappear altogether (Fig. 1d). The progressive loss of negatively staining membrane was often accompanied by a breakdown in sharpness of the interface between the two areas. Essentially identical results were obtained with 1, 10 or 100 µg ml⁻¹ cytochalasin B. Dimethylsulphoxide (DMSO) at equivalent dilution to that used to dissolve the cytochalasin B had no effect. The effect produced by both azide and cytochalasin B was observed at whatever stage in the protocol the drug was added, but was more marked if the drug was added before and during incubation with con A than if added after incubation. The effect was partially reversible, removal of cytochalasin B leading to an increased incidence of larger negative areas amongst eggs. Colcemid at doses from 0.36-36.00 µg ml⁻¹ had no effect on the staining pattern. Colcemid, cytochalasin B, DMSO and azide all caused rounding up of the eggs to spherical shapes, and cytochalasin B and DMSO, but not azide, caused a slight displacement of the metaphase spindle away from the surface membrane.

Newly fertilised eggs at pronuclear stages and earlier were also examined for reaction with con A. The egg was positive over the whole membrane, displaying a patchy staining pattern when incubated at 20 or 37 °C. The patchy, as distinct from smooth, staining pattern on fertilised eggs was induced by concentrations of con A one-fourth the minimum dilution effective for unfertilised eggs. An association was noted between a patchy staining pattern and the agglutination of eggs by con A, as both occurred more with fertilised than unfertilised eggs, with azide or cytochalasin B present and at 37 but not at 4 °C. The membrane overlying the midbody connecting the egg to the polar body was intensely positive, but the polar body was always either negative or had a small cup-shaped region of positive stain at its junction with the midbody (Figs 1e and 2).

These experiments demonstrate that the plasma membrane of the unfertilised mouse egg seems to be a mosaic of two distinct regions, one staining with fluorescent con A and constituting most of the egg's surface, the other on which little or no stain is detectable, overlying the second metaphase spindle and specifically isolated as the membrane of the second polar body. This pattern is susceptible to disorganisation by azide, which inhibits energy-dependent processes, and by cytochalasin B, which affects surface membrane activity and microfilament organisation. As it seems probable that energy-dependent microfilament activity is in part responsible for organising the contractile ring of the cleavage furrow which partitions the cell at division³, the mosaicism in the organisation of the surface con A-binding groups may reflect an underlying intracellular organisation dependent on microfilaments.

The nature of the membrane mosaicism is not clear from these experiments but could exist at either a structural or molecular level. A mosaicism of the underlying intracellular organelles is well established from ultrastructural studies, which have shown the cytoplasm in the spindle region to be relatively devoid of cortical granules, multivesicular bodies and membrane laminae². The mosaicism of con A binding seen at the light microscope level may be a molecular correlate of this polarity. The staining seems to be a genuine surface phenomenon and not the result of a selective pinocytosis in the positive area, as preincubation in azide and cytochalasin B caused extension of staining over the whole area rather than loss or reduction of positive staining. Organisation of the membrane of the positive area into microvilli may increase the fluorescence intensity either by increasing the effective density of con A-binding sites through corrugation of the surface or by effecting a reorganisation of binding sites within the membrane itself⁴. The intense staining of the midbody region is most probably caused by extensive surface corrugation⁵,

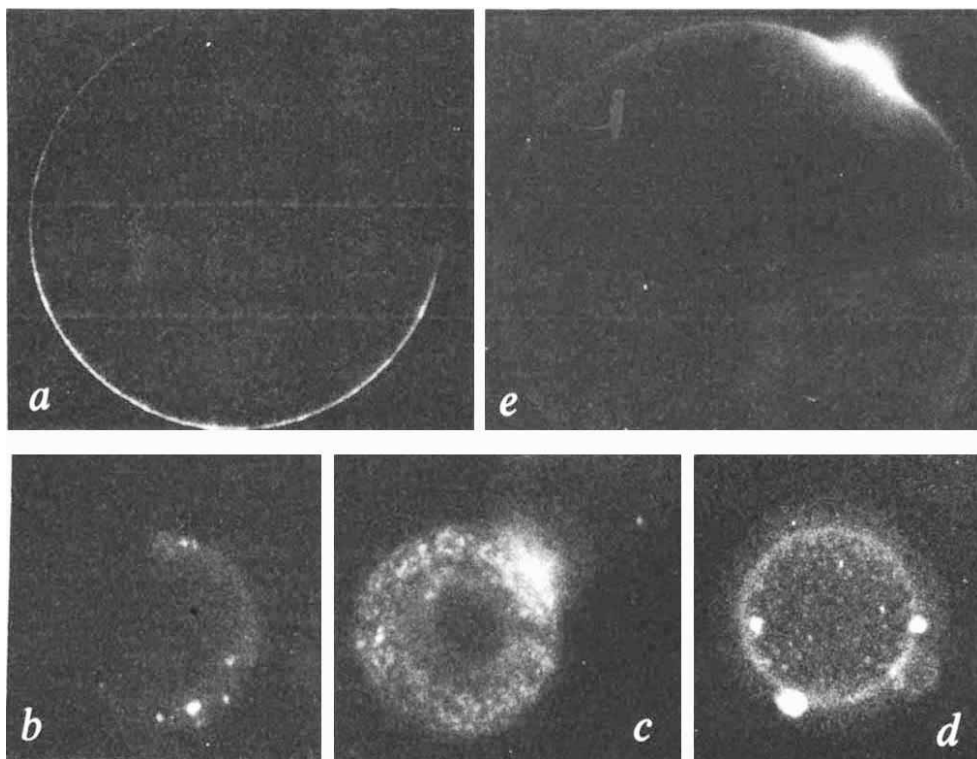


Fig. 1 Fertilised and unfertilised eggs were recovered from outbred CFLP and LACA mice within 2–3 h of superovulation. The cumulus cells were removed with hyaluronidase (Koch-Light 50 IU ml⁻¹ in phosphate-buffered saline with 10% polyvinylpyrrolidone), and the zona pellucida was removed with Pronase (0.5%, Tris-citrate buffer pH 7.0). Eggs were washed in medium PB1 (ref. 5) and used immediately. Eggs (5–10) were placed under oil in each well of a microtitre dish (Baird and Tatlock, tissue-typing slide catalogue 77 403/0522). Reagents were added by washing the eggs in each well with three changes of PB1 + reagent. Incubations were carried out at 4, 20 or 37 °C for 20–30 min. After incubation the eggs were washed in several changes of medium. A coverslip was applied and the eggs viewed on a Zeiss Photomicroscope (light source HBO200; excitation filters KP490 and 500, and LP455; barrier filters KP560 and LP520). Photographs were taken using Kodak recording film 2475. Fluorescein-labelled con A was obtained from Miles Laboratories and also synthesised in the laboratory. The activity was titrated and a final concentration of 50 µg ml⁻¹ was used in most experiments. α -Methyl-D-mannoside blocked staining. *a*, Unfertilised mouse egg incubated with fluorescein-labelled con A (25 µg ml⁻¹) at 20 °C for 30 min. Note absence of staining at one pole of egg ($\times 264$). *b*, As *a* but at lower magnification to show the clearly defined line of demarcation separating positive and negative areas ($\times 80$). *c*, Unfertilised egg stained with fluorescein-labelled con A in the presence of azide. Note how the size of the negative patch has contracted ($\times 80$). *d*, As *c* but showing an egg in which no negative area is visible ($\times 80$). *e*, Fertilised egg showing positive stain over whole surface of egg, intense stain of midbody but absence of stain in adjacent polar body ($\times 264$).

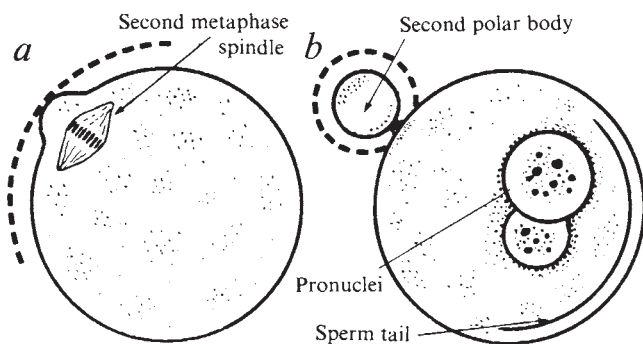


Fig. 2 Diagram indicating organisation of unfertilised (*a*) and fertilised eggs (*b*). Dotted outline indicates region of negative area.

and in preliminary ultrastructural studies we have found a reduced incidence of microvilli over the metaphase spindle. These aspects are being explored further at the ultrastructural level using ferritin-labelled con A.

Mosaicism at a molecular level could arise for several reasons. Masking of con A-binding residues in the negative area by neuraminidase-sensitive material has been excluded experimentally. Mannose-bearing oligosaccharides could be selectively excluded from the negative area. Alternatively, the oligosaccharides could be present but immobilised perhaps by way of microfilaments in such a way as to prevent cross linking by con A and their visualisation in patches¹, as evidently occurs elsewhere on the membrane. Note that in these studies the zonae pellucidae were removed by Pronase, which could have effects on the susceptibility to patching of the egg membrane.

Whatever the basis of the mosaicism, con A promises to be a useful probe of surface properties of the egg in different conditions. Such observations may also be relevant to the mechanisms of sperm-egg interaction. We have observed that sperm attachment *in vitro* to the negatively staining areas of zona-free unfertilised eggs is rare (M.H.J., and D.E., unpublished). The mosaicism of con A receptors may reflect an underlying membrane organisation, pre-

venting sperm attachment and fusion at a site at which extrusion of the second polar body may be impaired or abnormal extrusion of the fertilising sperm head may occur. Haploid or triploid states might thereby be avoided.

We thank John Marston for advice and Janis Ingram-Johnson for assistance. This work was supported by an MRC grant to M.H.J.

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Release of immunoreactive luteinising hormone-releasing hormone and thyrotrophin-releasing hormone from hypothalamic synaptosomes

THE hypophysiotropic hypothalamus regulates the secretion of the anterior pituitary gland through the production and release of specific peptide hormones. Luteinising hormone-releasing hormone (LHRH) and thyrotrophin-releasing hormone (TRH) are two such peptides which have been fully characterised and synthesised^{1,2}. Studies on intact animals have revealed the importance of various neurotransmitters and hormones in the modulation of hypothalamic LHRH and TRH secretion but little is known of the cellular mechanisms involved in such control, especially at the neurosecretory nerve terminal. We have described^{3,4} the use of nerve endings (synaptosomes) isolated from the mammalian hypothalamus to investigate factors which influence the release of corticotrophin-releasing factor (CRF). As this factor is uncharacterised, a major limitation of this work has been the use of bioassay techniques to measure indirectly corticotrophin-releasing activity. In the study described here radioimmunoassays have been used to measure the release of immunoreactive LHRH and TRH from synaptosomes prepared from rat and sheep hypothalamus, and we report the influence of neurotransmitters and steroid hormones on these processes.

Nerve endings were isolated from hypothalami (600–800 mg, wet weight, sheep; 30–50 mg, rat) by the method described previously⁴ and suspended in Krebs bicarbonate medium containing 10 mM glucose at a concentration of 1 and 3 hypothalamic equivalents per ml for sheep and rat, respectively. In some experiments sheep hypothalami were dissected into three regions: the median eminence (50–100 mg); a more dorsal, medial periventricular region (250–300 mg) and the remainder of the hypothalamus. These fragments were collected and treated separately. Samples (1 ml) of synaptosome suspensions were incubated for 30 min at 37 °C in capped glass vials. Electrical field stimulation was carried out during the last 20 min of incubation by passing alternating square wave pulses through concentric gold ring electrodes immersed in the synaptosome suspensions. The parameters of this electrical stimulation have been described previously⁴. Potassium stimulation was achieved by increasing the potassium concentration of the incubation medium to 60 mM during the last 10 min of incubation. In further experiments neurotransmitters or steroid hormones were added directly to the vials in 20 µl of incubation medium. After incubation the synaptosomes were removed by centrifugation, and the supernatants were rapidly frozen immediately and stored at –20 °C for 1–5 d before measurement of the LHRH and TRH content by radioimmunoassays, which have been described in detail elsewhere^{5,6}.

Synaptosomes isolated from whole sheep hypothalami released both LHRH and TRH to the medium (Table 1). Unstimulated preparations released almost fifteen times more TRH than LHRH, estimated on a molar basis, whereas electrical stimulation produced a sevenfold increase in LHRH and a fivefold increase in TRH in the medium. In contrast, depolarising concentrations of potassium had no significant effect on the release of either hormone. This difference in response to the two stimuli is surprising: it is possible either that the transitory depolarisations produced by electrical pulses are a more effective stimulus than the tonic depolarisation produced by potassium, or alternatively that elevated potassium levels may in some way stimulate the reuptake or degradation of the

Table 1 Release of immunoreactive LHRH and TRH by synaptosomes isolated from sheep hypothalamus

Test	LHRH	TRH
Control	271 ± 24 (11)	1,536 ± 182 (14)
60 mM K ⁺	289 ± 23 (12)	1,617 ± 148 (9)
Electrical field stimulation	2,018 ± 696 (12)*	7,217 ± 1,060 (12)†

Values represent pg of immunoreactive hormone (mean ± s.e.m.; number of experiments shown in parentheses) released by 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) during 30 min incubation in Krebs bicarbonate medium at 37 °C. Electrical field stimulation and potassium stimulation were for the last 20 min and 10 min respectively. Student's *t* test: **P* < 0.02, †*P* < 0.001.

hormones released to the medium. Synaptosomes prepared from whole hypothalami of sheep were incubated in the presence of a range of concentrations of neurotransmitters known to be present in the mammalian hypothalamus, particularly in the median eminence (Table 2). Dopamine at concentrations of 10⁻¹⁰ M or more stimulated the release of LHRH but was without effect on TRH release. In contrast, 5-hydroxytryptamine (5-HT) at 10⁻⁸ M had no effect on LHRH release but significantly inhibited the release of TRH. Acetylcholine, noradrenaline and adrenaline were without effect on either LHRH or TRH release.

Nerve endings prepared from the three dissected regions of the sheep hypothalamus were also incubated and electrically stimulated or tested with dopamine (10⁻⁸ M) and the results are shown in Table 3. The levels of LHRH and TRH released from control and electrically stimulated preparations from the median eminence resembled those obtained with preparations derived from whole hypothalami. The incubation of median eminence synaptosomes in the presence of dopamine stimulated the release of both LHRH and TRH. The absence of an effect of dopamine on TRH release in preparations from the whole hypothalamus may thus reflect the heterogeneous nature of the synaptosome population and the possibility that inhibitory neurotransmitters or other factors can block the stimulation induced by dopamine.

Synaptosomes prepared from the central periventricular region and the remainder of the hypothalamus released low

Table 2 Effect of neurotransmitters on the release of immunoreactive LHRH and TRH by synaptosomes isolated from sheep hypothalamus

Neurotransmitter	Concentration (M)	LHRH	TRH
Control		271 ± 24 (11)	1,536 ± 182 (14)
Dopamine	10 ⁻¹²	253 ± 50 (5)	1,752 ± 180 (5)
	10 ⁻¹⁰	555 ± 130 (10)*	1,361 ± 130 (9)
	10 ⁻⁸	537 ± 180 (6)	1,356 ± 184 (6)
	10 ⁻⁶	560 ± 113 (3)*	1,181 ± 335 (3)
Noradrenaline	10 ⁻¹²	210 ± 16 (7)	1,967 ± 1,250 (3)
	10 ⁻¹⁰	271 ± 46 (9)	1,320 ± 259 (5)
	10 ⁻⁸	308 ± 22 (6)	1,253 ± 64 (4)
	10 ⁻⁶	243 ± 41 (3)	1,327 ± 156 (4)
5-HT	10 ⁻¹²	201 ± 7 (3)	1,968 ± 143 (3)
	10 ⁻¹⁰	234 ± 26 (6)	1,918 ± 172 (6)
	10 ⁻⁸	273 ± 52 (3)	666 ± 82†
	10 ⁻⁶	207 ± 23 (3)	660 ± 77†
Acetylcholine	10 ⁻⁸	253 ± 40 (6)	1,600 ± 206 (3)
Adrenaline	10 ⁻⁸	289 ± 37 (4)	1,708 ± 196 (4)

Values represent pg of immunoreactive hormone (mean ± s.e.m.; number of experiments shown in parentheses) released by 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) during 30 min incubation in Krebs bicarbonate medium at 37 °C. Neurotransmitters were added in 20 µl of medium at the start of each incubation. Student's *t* test: **P* < 0.05, †*P* < 0.001.

Table 3 Release of immunoreactive LHRH and TRH by synaptosomes isolated from different regions of the sheep hypothalamus

Test	LHRH			TRH		
	Median eminence	Periventricular hypothalamus	Remainder of hypothalamus	Median eminence	Periventricular hypothalamus	Remainder of hypothalamus
Control	169±20 (9)	64±30 (6)	47±10 (6)	1,222±223 (9)	853±307 (6)	720±126 (6)
Electrical stimulation	601±105 (9)*	153±40 (7)§	95±20 (8)§	5,044±909 (9)*	3,080±494 (7)†	3,260±680† (5)
Dopamine (10 ⁻⁸ M)	565±154 (11)‡	46±6 (8)	39±9 (8)	4,854±927 (11)†	1,213±200 (8)	1,115±130 (7)

Values represent pg of immunoreactive hormone (mean±s.e.m., number of experiments in parentheses) released to 1 ml of a suspension of hypothalamic synaptosomes (equivalent to one hypothalamus) prepared from three different regions as described in the text and incubated for 30 min in Krebs bicarbonate medium at 37 °C. Electrical stimulation was carried out during the last 20 min of incubation while dopamine was added at the start of the incubation period: Student's *t* test: **P* < 0.001, †*P* < 0.005, ‡*P* < 0.02 §*P* < 0.05—tested against control results for each preparation.

levels of LHRH whereas the amounts of TRH were higher and comparable with those released from median eminence preparations. Electrical stimulation caused release of both hormones and the responses were of similar magnitude to those obtained with synaptosomes prepared from the median eminence. In contrast with the latter preparation, dopamine added to synaptosomes obtained from the periventricular region or remainder of the hypothalamus did not result in the increased release of LHRH or TRH. This may indicate that the LHRH and TRH-containing nerve endings which terminate outside the median eminence do not possess receptors for dopamine, or that these preparations contain an inhibitory factor(s) which may block the response to this transmitter.

The inhibitory effect of 5-HT on TRH release and the stimulatory effect of dopamine on LHRH and TRH release from synaptosomes prepared from the median eminence are consistent with findings obtained in the whole animal^{7,8}, and with the isolated hypophysiotropic hypothalamus incubated *in vitro*⁹⁻¹¹. These results, together with our finding³ that acetylcholine stimulates and dopamine inhibits the release of CRF from hypothalamic nerve endings, suggest that the neurotransmitters present in the median eminence have a specific role in modulating the release of hypophysiotropic hormones. The extent and physiological significance of such control mechanisms at the neurosecretory nerve terminal remain to be established but further evidence for integration at this level has been obtained from experiments in which the effects of gonadal steroids on the release of LHRH from synaptosomes prepared from the rat hypothalamus have been investigated.

Adult female Sprague-Dawley rats (250 g body wt) were treated with 17- β -oestradiol and progesterone, alone and in combination, administered as a microparticulate suspension in the drinking water (10 μ g ml⁻¹) for 20 h (average ingested dose 150 μ g per rat) before killing, removal of the hypothalami and preparation of synaptosomes. The same steroids were tested *in vitro* at a concentration of 10⁻⁸ M for a direct

action on hypothalamic synaptosomes prepared from untreated rats. In all cases tested the release of TRH was unaffected by these procedures. Table 4 shows that synaptosomes prepared from the rat hypothalami released much smaller amounts of LHRH than the sheep preparations (14 pg per hypothalamic equivalent compared with 271 pg) although these amounts are in proportion to the weights of original tissue. Pretreatment with oestradiol or progesterone *in vivo* resulted in a two- to threefold increased release of LHRH, whereas simultaneous pretreatment with both hormones produced a marked inhibition. When added directly *in vitro*, oestradiol (10⁻⁸ M) alone had no apparent effect on the nerve endings, although progesterone (10⁻⁸ M) alone and in the presence of oestradiol resulted in an increased release of LHRH. The rats used in these experiments were mature females in all stages of the oestrous cycle and the results can only confirm the complexity of interactions between gonadal steroids and the synthesis and release of LHRH. The *in vitro* results, however, suggest that at least part of the feedback effects of gonadal steroids may occur at the neurosecretory nerve ending; an effect analogous to our previous finding³ that glucocorticoids inhibit the release of CRF from hypothalamic synaptosomes. To clarify further the effects of gonadal steroids *in vitro* shown in Table 4, the effects of 17- β -oestradiol and progesterone on the basal release of LHRH from hypothalamic synaptosomes prepared from female rats taken from specific stages of the oestrous cycle are now being examined. Like the actions of neurotransmitters, the effects of gonadal steroids administered *in vitro* seem to be specific. Thus progesterone increases the release of LHRH, but has no effect on TRH release and at high levels inhibits the release of CRF induced by electrical stimulation. These findings support our view³ that steroids may influence neural processes through mechanisms which do not involve the classical nuclear receptor for such hormones. Studies are in progress to establish whether these effects could be mediated through the action of cyclic nucleotides and mechanisms involving protein synthesis.

This work was supported through an MRC programme grant to J.A.E. We thank Mrs J. Pennington for technical assistance.

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Table 4 Effect of gonadal steroids on release of immunoreactive LHRH by synaptosomes isolated from the female rat hypothalamus

Test	LHRH	
	<i>In vivo</i> pretreatment	Added <i>in vitro</i>
Oestradiol	121±35 (6)*	34±18 (5)
Progesterone	92±16 (5)†	135±48 (6)*
Oestradiol + progesterone	17±4 (5)‡	118±42 (3)
Untreated controls	43±7 (8)	

Values represent pg of immunoreactive LHRH (mean±s.e.m., number of experiments in parentheses) released by 1-ml suspension of hypothalamic synaptosomes (equivalent to three hypothalami) during 30 min incubation in Krebs bicarbonate medium at 37 °C. For pretreatment (*in vivo*) rats were administered steroid (10 μ g ml⁻¹) in drinking water for 20 h before isolation of synaptosomes. Steroids (10⁻⁸ M) were added directly to synaptosome suspensions for experiments (*in vitro*). Student's *t* test, comparison with untreated control values: **P* < 0.05, †*P* < 0.02, ‡*P* < 0.005.

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Isolation and structure of urogastrone and its relationship to epidermal growth factor

THE clinical observation of the very low incidence of peptic ulceration during pregnancy led to the finding that extracts of the urine of pregnant women had a beneficial effect on experimental ulcers in dogs¹. It was later reported that not only pregnancy urine but also that from all females and males contained a potent inhibitor of gastric acid secretion^{2,3}. Sandweiss⁴ named the anti-ulcer factor anthelone and regarded it as having a therapeutic effect on ulcers without depressing gastric acid secretion. The antisecretory agent was thought to be a separate entity and was called urogastrone because its actions resembled those of the postulated duodenal hormone enterogastrone⁵. Subsequent work amply demonstrated urogastrone action, but the existence of anthelone as a separate entity remained less well established. To establish the true nature of these agents, however, and in particular their possible role in the therapeutic control of peptic ulceration it was necessary to make a full chemical identification. Many attempts have been made to isolate urogastrone, and probably the most highly purified sample on record was that obtained by Gregory⁶. This was described as a combination of a golden yellow fluorescent pigment and a protein of relatively low molecular weight. Others have provided additional evidence of a protein structure⁷ but some recent work has led to the suggestion that urogastrone is a high-molecular weight glycoprotein^{8,9}. Nevertheless its exact nature, source¹⁰ and physiological role have remained unknown. Gastric inhibitory effects have also been shown by extracts of animal urine but studies were undertaken on the nature of urogastrone from more readily available human urine.

Highly purified samples of urogastrone from normal male urine have now been obtained by means of a twelve-stage purification process involving ion exchange, partition and gel chromatography¹¹. There seems to be more than one inhibitor of gastric acid secretion in urine but the main active components were eventually obtained in yields of less than 1 mg per 1,000 l of urine. Two closely related products were isolated and for these the original name of urogastrone has been used. The two urogastrones were shown to be water-soluble polypeptides of relatively low molecular weight and the difference between them could be shown by various physical techniques including acrylamide gel electrophoresis (Fig. 1). They caused an intense inhibition of gastric acid secretion in cats and dogs (when tested as described¹² for less pure material obtained previously) and seemed to be biologically indistinguishable. Because of the small amounts of material available, the two peptides were sometimes used together. Doses as low as 0.25 µg kg⁻¹ produced 60–80% inhibition in Heidenhain pouch dogs stimulated to near maximal levels of acid secretion with pentagastrin or histamine. Work with less pure material had established that at doses producing strong inhibition of acid secretion, urogastrone did not affect other secretions such as pancreatic, biliary or salivary secretion and the effects seemed to be quite specific for the stomach. It did not affect blood pressure, pulse rate or body temperature¹³. Also, it did not seem to be inhibiting acid secretion by causing restriction of mucosal blood flow^{13,14}. Urogastrone has been shown to be active in a number of species and, in man, doses of 0.25 µg kg⁻¹ strongly inhibit gastric secretion induced by different stimuli¹⁵. Acid secretion was also

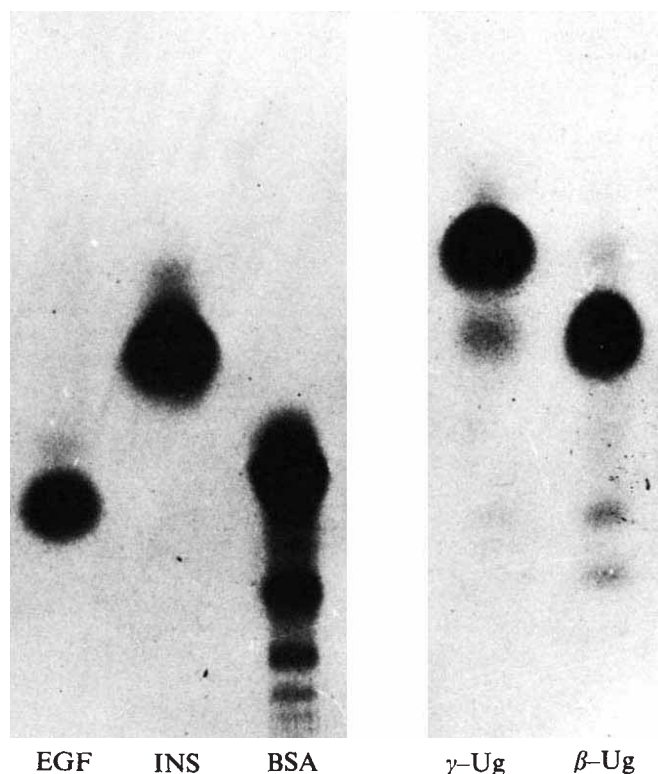
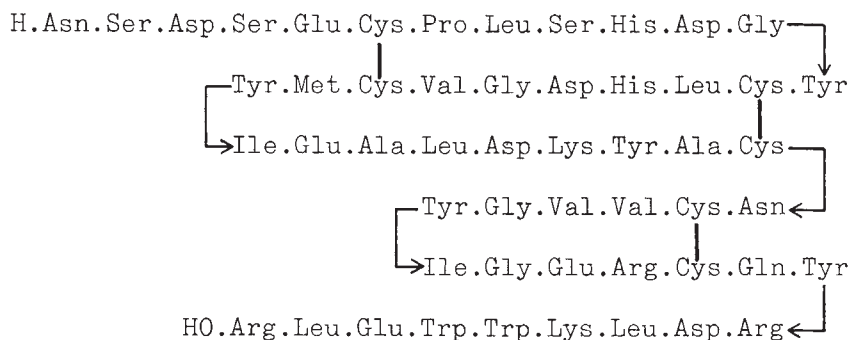


Fig. 1 Thin-layer acrylamide gel electrophoresis at pH 8.9 in which the two urogastrones (β-Ug, γ-Ug) and epidermal growth factor (EGF) are compared with control samples of bovine insulin (INS) and bovine serum albumin (BSA) stained with amido black. The lower edge of the photograph indicates the origin and the upper edge the distance moved by bromophenol blue to the anode.

reduced in patients with Zollinger Ellison syndrome who had massive gastric hypersecretion¹⁶.

The two isolated polypeptides, designated β and γ urogastrone, were found to consist of 53 and 52 amino acid residues respectively. The full structures of these peptides have now been established (Fig. 2) and it was found that the difference resided only in the C-terminal arginine residue which was absent in the γ peptide (H. G., and B. Preston, unpublished). The amino acid sequence was established mainly by degradation of the S-carboxymethylated or S-carboxamidomethylated peptides using trypsin, chymotrypsin, thermolysin or partial acid hydrolysis followed by dansyl Edman degradation of the isolated fragments. The protease derived from *Armillaria mellea* was of particular value, giving specific bond cleavage on the amino side of lysine^{17,18} and providing three fragments from urogastrone derivatives. The positions of the disulphide bonds were established by partial acid degradation of the intact urogastrone molecule.

When this sequence was established it seemed to be quite different from any other known structure and from known gastrointestinal hormones in particular. But a structure has recently been presented for mouse epidermal growth factor (EGF)¹⁹, a polypeptide isolated from the submaxillary glands of male mice which seem to be quite a rich source of biologically active peptides. Early work with this material showed that it possessed the remarkable property of causing premature eye opening when injected daily into newborn mice²⁰ and it has subsequently been shown to stimulate the growth of epithelial cell tissue in a variety of preparations²¹. EGF was isolated as 53 and 51 residue peptides and the published structures showed interesting similarities to urogastrone. Epidermal growth factor was therefore isolated by Cohen's procedure with minor variations and the material obtained had physical properties as described²². The behaviour of EGF on thin-layer acrylamide gel

Fig. 2 The amino acid sequence of β -urogastrone.

electrophoresis was compared with the two urogastrones (Fig. 1); it moves more slowly to the anode at pH 8.9 than the two urogastrones which differ by the single arginine residue. The potency of this preparation was confirmed by its action on newborn mice (Alderley Park Strain). EGF given as subcutaneous injections of 5 μg in 25 μl saline d⁻¹ caused the eyes to open on day 10 compared with day 13 in the controls²³. It was found subsequently that this material would produce strong inhibition of gastric acid secretion in rats and dogs when given intravenously against exogenous stimuli²³. But the doses of EGF required to induce eye opening ranged from over 300 $\mu\text{g kg}^{-1}\text{d}^{-1}$ initially to about 100 $\mu\text{g kg}^{-1}$, whereas inhibition of acid secretion was obtained with less than 0.5 $\mu\text{g kg}^{-1}$ in dogs and less than 10 $\mu\text{g kg}^{-1}$ in rats. The time course of inhibition was indistinguishable from that obtained by intravenous injections of urogastrone¹². Maximum inhibition was obtained 15–30 min after injection and secretion then gradually returned to plateau levels after some 90 min. Longer periods of inhibition could be obtained by giving the peptides subcutaneously at rather higher doses. It seemed, therefore, that EGF had biological actions similar to those established for urogastrone and so urogastrone was examined to see whether it would produce the known effects of EGF on newborn mice. At the established dose levels of 5 μg per mouse d⁻¹ it was found that urogastrone would also induce eye opening on day 10 compared with day 13 for the controls (J. M. Bower, unpublished). Although the full spectrum of EGF actions has not been studied there is substantial overlap in the properties of the two polypeptides.

The two peptides show remarkable structural similarities

(Fig. 3). Of the 53 amino acid residues comprising the longer urogastrone and EGF molecules 37 are common to both peptides. The six cysteines occupy the same relative positions and the three disulphide bonds are formed in the same direction. The 16 variable residues occur at intervals along the chains and of these, 14 are interpretable as single base changes in the coding triplets. The two additional pairs are at position 28—Ser, Lys—and position 44—Thr, Tyr. The longest sequences common to both peptides consist of five residues but one of these—the interesting looking C-terminal pentapeptide—does not seem to be necessary for inhibitory activity^{24,25}.

The overall number of changes is perhaps greater than found typically in hormones with similar functions from different species²⁶. There are only four changes in the 51 residues of the insulins of mice and men although guinea pig insulin shows 16 changes when compared with that of man. On the other hand, there are hormone families, the gastrointestinal group of secretin, glucagon, VIP and GIP for example, in which differing biological actions are associated with molecules having common structural features. In this case, secretin differs from glucagon by 14 of the 29 residues²⁷. The structural relationship of urogastrone and epidermal growth factor, however, is supported by apparently identical biological actions.

The salivary glands of species other than the mouse are not a good source of the growth factor²⁰ and only the male mouse provides substantial quantities. Females can be made to produce much larger amounts of EGF, however, as a result of androgen treatment²⁸. This sexual dimorphism does not normally apply to urogastrone and levels in urine are very similar for the human male and non-pregnant female¹⁰. Interestingly, the only

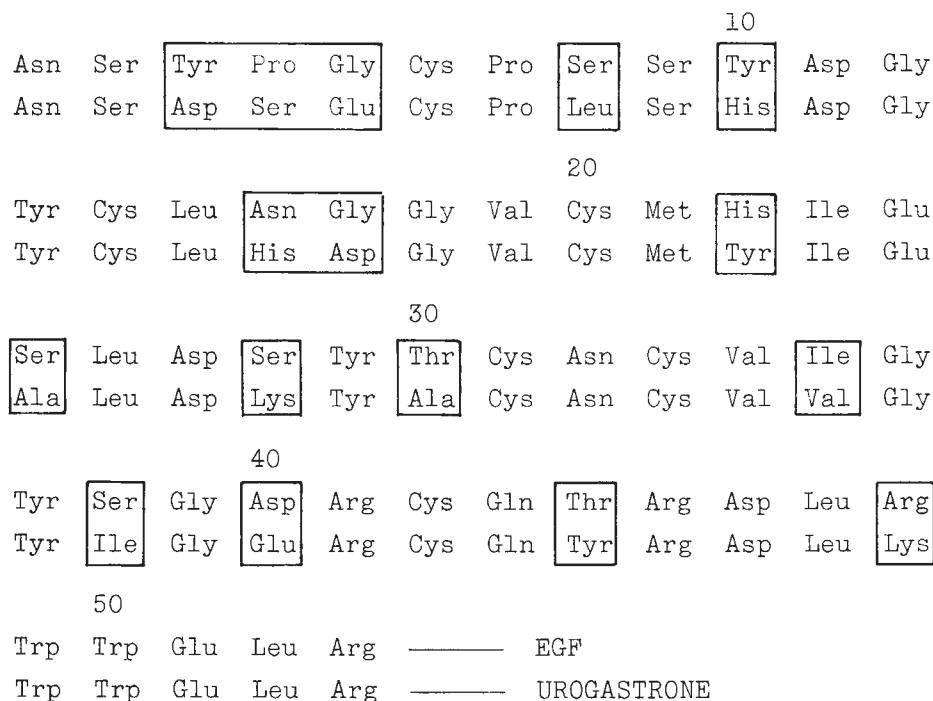


Fig. 3 Comparison of the amino acid sequences of human urogastrone and mouse epidermal growth factor with the changed residues enclosed.

Table 1 Amino acid composition of urogastrone compared with human and mouse epidermal growth factor

Amino acid	β -Urogastrone	Human EGF ³⁰	Mouse EGF ¹⁹
Lys	2	3	0
His	2	2	1
Trp	2	1	2
Arg	3	2	4
Asp	7	7	7
Thr	0	0	2
Ser	3	3	6
Glu	5	5	3
Pro	1	2	2
Gly	4	5	6
Ala	2	2	0
Half-Cys	6	6	6
Val	3	2	2
Met	1	1	1
Ile	2	2	2
Leu	5	4	4
Tyr	5	2	5
Phe	0	0	0
Total	53	49	53

indications of differences in urogastrone excretion lie in the many reports of higher urinary levels during pregnancy, when oestrogen and progesterone levels are raised. A recent study with a mouse EGF immunoassay showed that blood levels of an immunoreactive species could be demonstrated during the early stages of pregnancy in humans but none could be detected in the male or non-pregnant female²⁹. A sensitive radioimmunoassay for human urogastrone as well as EGF has been developed (H. G., J. E. Holmes, and I. R. Willshire, unpublished) and accurate determinations of urogastrone and possible EGF in various human conditions are being made.

Recently, a human epidermal growth factor has been isolated from human pregnancy urine and a total amino acid composition has been given³⁰. These figures are given in Table 1 together with mouse EGF and are compared with the ratios for β -urogastrone. Although there are differences in several of the figures, those for human EGF are not far removed from the urogastrone ratios and may be indicative of two closely related polypeptides. Because the problems of obtaining accurate amino acid ratios with small amounts of material are well known, however, it is probable that urogastrone and human epidermal growth factor are one and the same.

It is worth recalling the definition that Sandweiss applied to anthelone over 30 years ago—that it produced a beneficial effect on experimental ulcers by promoting fibroblastic proliferation and epithelialisation of the mucosa³¹. It seems that the gastric inhibitory polypeptide urogastrone may also have the epithelial growth characteristics once ascribed to anthelone as a separate entity and work now in progress will show whether it may be of value as a therapeutic agent in the treatment of duodenal ulceration.

I thank colleagues who have been involved in different aspects of the work on urogastrone and in particular Mr I. Willshire, Mrs B. Preston, Dr J. Raventos, Mrs E. Haworth, Dr L. Gerring, Miss J. Bower and Mr W. Broadbent.

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Received July 18; accepted August 1, 1975.

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Incorporation of GMP into specific tRNA molecules by extracts of Ehrlich ascites tumour cells

ENZYMATIC activities for synthesising four ribohomopolymers, including poly(G), have been found in rat liver, and all are stimulated by RNA^{1,2}. Recently two poly(G) polymerases were isolated from plant cells; one requires poly(G) as a primer³ and the other denatured DNA⁴. We report here the isolation of an enzyme from Ehrlich ascites tumour, which catalyses the incorporation of GMP from GTP into an acid-insoluble product. This enzyme requires specific tRNAs as primers. Our data suggest that the tRNAs which can serve as primers are specific to tumours or tissues infected with tumour viruses.

The GTP incorporating enzyme activity we studied was

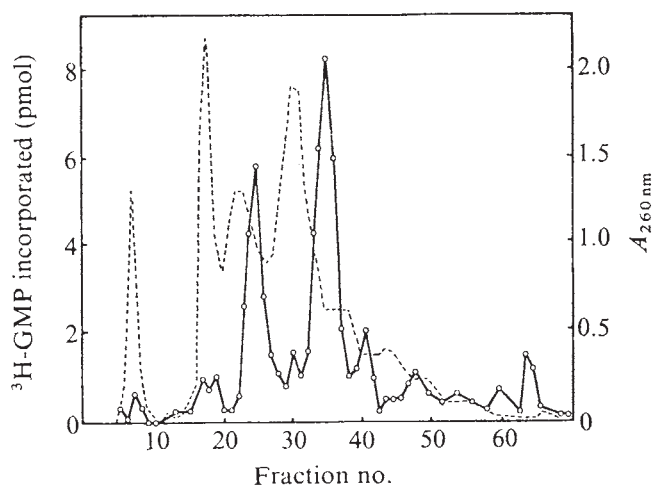


Fig. 1 Primer activity of various tRNAs from Ehrlich ascites tumour. About 3.8 ml of tRNA solution (2.4 mg ml⁻¹) containing 0.25 M NaCl was applied to a 0.6 × 90-cm RPC-5 (ref. 6) column, previously equilibrated with buffer (0.01 M Tris-HCl, pH 7.5, 0.01 M Mg(CH₃COO)₂, 0.0003 M 2-mercaptoethanol, 0.4 M NaCl). A 400-ml linear gradient of 0.5–1.0 M NaCl was generated and pumped (about ~20 kg cm⁻²) through the column at about 0.3 ml min⁻¹. 4.7-ml fractions were collected. This experiment was performed at the National Cancer Center Research Institute, through the kindness of Dr S. Nishimura. The absorbance at 260 nm of each fraction was measured (dotted line). Primer activity of each fraction was measured by adding 0.02 ml of each fraction into the standard reaction mixture containing 30 µg of enzyme protein (continuous line).

Table 1 Effects of various nucleic acids offered as primers

Primer	(μ g)	3 H-GMP incorporated (pmol)	Primer	(μ g)	3 H-GMP incorporated (pmol)
Experiment 1					
Ehrlich ascites tumour			Mouse spleen RNA	10	1.0
Whole RNA	10	13.6	liver RNA	10	1.3
tRNA	2.5	13.5	Rat spleen RNA	10	<0.5
rRNA	10	0.5	liver RNA	10	0.5
<i>E. coli</i>			MS2 phage RNA	10	<0.5
Whole RNA	10	25.9	GA phage RNA	10	<0.5
tRNA	2.5	27.4	Q β phage RNA	10	<0.5
rRNA	10	3.1	SP phage RNA	10	<0.5
Methionine tRNA _f	5	< 0.5	FIC phage RNA	10	<0.5
Methionine tRNA _m	5	< 0.5	TMV RNA	10	2.0
Valine tRNA _i	5	< 0.5	Calf thymus DNA (native)	10	<0.5
Phenylalanine tRNA	5	< 0.5	(denatured)	10	<0.5
Glutamic acid tRNA _{ii}	5	< 0.5	Experiment 2	(nmol)	
Serine tRNA _i	5	< 0.5	<i>E. coli</i> tyrosine tRNA _{ii}	0.01	35.6
Serine tRNA _{iii}	1.3	19.6	poly(A)	0.1	2.3
Leucine tRNA _{ii}	2.5	15.1	poly(C)	0.1	0.5
Tyrosine tRNA _i	0.65	38.8	poly(U)	0.1	1.3
Tyrosine tRNA _{ii}	0.65	42.6	poly(G)	0.1	1.3

The standard reaction mixture contained in a total volume of 0.25 ml, 22 μ mol of Tris-HCl, pH 8.0, 3.2 μ mol of MgCl₂, 0.02 μ mol of 2-mercaptoethanol, 0.1 μ mol of 3 H-GTP (2×10^6 c.p.m. per 0.1 μ mol), 30 μ g (experiment 1) or 25 μ g (experiment 2) of enzyme protein and each of the indicated RNAs as a primer were added. After incubation at 36 °C for 20 min, the cold 5% trichloroacetic acid-insoluble materials were collected on membrane filters, and the radioactivity of the dried filters was counted. Male mice (ddY) were inoculated intraperitoneally with 10^6 cells per mouse of Ehrlich ascites tumour cells, supplied by Dr D. Mizuno (Tokyo University), and after 6 to 8 d, cells were collected and used for preparation of enzyme and RNA. RNAs were extracted from *E. coli* cells, Ehrlich ascites tumour cells, and tissues of mouse and rat by cold sodium dodecyl sulphate-phenol methods¹⁰. tRNA and rRNA fractions were prepared by 5–30% glycerol gradient centrifugation of whole RNA. Ten purified *E. coli* tRNAs were supplied by Dr S. Nishimura (National Cancer Center Research Institute). Q β phage RNA was prepared as described by Haruna and Spiegelman¹¹. Other phage RNAs were gifts of Dr T. Shiba (Mitsubishi-Kasei Institute of Life Science). TMV RNA was given by Dr Y. Okada (Tokyo University).

found in the RNA-dependent RNA polymerase fraction⁵ of Ehrlich ascites tumour cells, and we have further purified the enzyme about 30-fold (see legend to Table 2). The whole RNA fraction extracted from these cells was active as a primer (Table 1). When the whole RNA was fractionated, tRNA was about four times as active as the whole RNA, while rRNA was almost inactive. To determine whether all the molecular species of tRNA were active as primers the tRNA fraction of Ehrlich ascites tumour cells was further separated by a reversed-phase chromatography, RPC-5 (ref. 6). Figure 1 shows clearly that some specific tRNAs were quite active as primers and others were inactive. The primer activity of tRNA in the fraction 35 of Fig. 1 was seven times that of unfractionated tRNA. Further separation of the tRNAs and ultimately the sequence analysis of each purified species will be necessary to identify the molecular species of the tRNAs which are active as primers.

To obtain information about the common structural characteristics of the primer tRNAs, we tested the primer activity of various RNAs extracted from *Escherichia coli* (Table 1), since the complete nucleotide sequences of about ten *E. coli* tRNAs have been determined^{7,8}. The whole RNA extracted from *E. coli* was active, and tRNA was also quite active but rRNA was almost inactive. Among the ten purified tRNAs of *E. coli*, only leucine tRNA_{ii}, serine tRNA_{iii}, tyrosine tRNA_i and tyrosine tRNA_{ii} were active as primers. It is interesting that all active tRNAs possess the S-region in their secondary structure, and were eluted at the end of neutral DEAE-Sephadex A-50 chromatography⁹. It should be noted that serine tRNA_i, which was inactive as a primer, was eluted at the top region of DEAE-Sephadex A-50 chromatography, although it possesses the S-region. Thus the primer activity of tRNA molecules may be related to their structural properties which determine their positions of elution in DEAE-Sephadex A-50 chromatography.

The enzymatic reaction showed an absolute requirement for primer RNA (Table 2, experiment 1). The reaction was neither inhibited by creatine phosphate and creatine phosphokinase nor by inorganic phosphate, but was inhibited by inorganic pyrophosphate. This suggests that guanosine tri-

phosphate is the actual substrate. The three ribonucleoside triphosphates other than GTP were not required for incorporation of GMP. The enzyme required magnesium or manganese ions, the optimum concentration being 7–14 mM and about 5 mM, respectively, and it also required 2-mercaptoethanol, its optimum concentration being 0.08 mM. *E. coli* tyrosine tRNA_{ii} lost all its primer activity after oxidation by periodate or after digestion with RNase T₁. When ribonucleoside triphosphates other than GTP were offered as substrates, no enhancement of incorporation was observed when tRNA was added (Table 2, experiment 2).

Various nucleic acids other than RNAs extracted from Ehrlich ascites tumour and *E. coli* were tested and found to be inactive as primers (Table 1). Moreover, none of these inactive RNAs was inhibitory to the enzyme reaction, as tested with *E. coli* tyrosine tRNA_{ii} as the primer.

The radioactive product synthesised by the enzyme with tyrosine tRNA_{ii} as a primer had a molecular size of about 4S as determined by glycerol gradient centrifugation. Treatment with alkali and RNases, such as RNase T₂, RNase T₁, RNase A and PDase (venom phosphodiesterase), made the radioactive product acid soluble. The radioactive substances in the hydrolysates as identified by cellulose thin-layer chromatography, Dowex-1 or DEAE-Sephadex A-25 column chromatography were as follows: alkaline hydrolysis, 2'-GMP and 3'-GMP; RNase T₂ treatment, 3'-GMP; RNase T₁ treatment, 3'-GMP and oligonucleotides; RNase A, oligonucleotides with chain lengths of more than two; and PDase treatment, 5'-GMP. The molecular ratio of incorporated GMP to the primer tyrosine tRNA_{ii} reached a maximum of 10–15. These preliminary analyses of the products indicated that the radioactivity existed in GMP residues which were attached covalently to the primer tRNA by phosphodiester linkages. Determination of the structure of the radioactive product is now in progress.

The presence of primer RNA in Ehrlich ascites tumour and the absence in normal mouse liver (Table 1) prompted us to search for primer RNAs in other tissues and tumours of mouse. RNAs extracted from mouse spleen and kidney, as well as liver, were inactive as primers while RNAs extracted from tissues of mice infected with Friend virus or transplanted

Table 2 Characterisation of the reaction

Assay condition	³ H-GMP incorporated (pmol)
Experiment 1 Complete	44.8
– RNA	< 0.5
– Enzyme	< 0.5
– Enzyme + heated enzyme	1.2
+ ATP, UTP, CTP (0.1 μmol each)	41.9
+ Creatine phosphate (1 μmol), creatine phosphokinase (10 μg)	44.7
+ Trypsin (20 μg)	10.0
+ RNase A (10 μg)	< 0.5
+ DNase (10 μg)	44.6
+ Inorganic phosphate (1 mM)	51.8
(10 mM)	49.8
+ Inorganic pyrophosphate (1 mM)	48.4
(4 mM)	29.6
(8 mM)	19.3
Experiment 2	(μg)
³ H-GTP + tyrosine tRNA ₁₁	1.3
+ glutamic acid tRNA ₁₁	2.5
³ H-ATP + tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5
³ H-UTP + tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5
³ H-CTP + tyrosine tRNA ₁₁	2.5
+ glutamic acid tRNA ₁₁	2.5

Heated enzyme was prepared by heating at 60 °C for 10 min. The complete reaction mixture was as described in the legend to Table 1, except that 30 μg of enzyme protein and 0.33 μg of purified *Escherichia coli* tyrosine tRNA₁₁ as a primer were added (experiment 1). The reaction mixtures in experiment 2 were similar to those in experiment 1 with these alterations: 0.1 μmol each of tritiated ribonucleoside triphosphates (2 × 10⁶ c.p.m. per 0.1 μmol) was used as substrate; the amount of enzyme was 11 μg, and purified *E. coli* tRNA was added as a primer. In experiment 2, the radioactivity for the sample without added tRNA was subtracted from each value. Washed Ehrlich ascites tumour cells were suspended in lysis buffer (0.1 M Tris-HCl, pH 7.4, 0.01 MgCl₂, and 0.0005 M 2-mercaptoethanol) containing DNase (5 μg ml⁻¹), and frozen and thawed once. After homogenisation for 40 s, the mixture was stirred at 4 °C for 30 min and EDTA was added to a final concentration of 0.01 M. The homogenate was then centrifuged at 105,000g for 2 h. From the supernatant the 35–50% (NH₄)₂SO₄ precipitate was collected and dissolved in lysis buffer, and the appropriate amount of protamine sulphate was added to remove nucleic acids. The 60% (NH₄)₂SO₄ precipitate was collected and dissolved in the standard buffer (0.01 M Tris-HCl, pH 7.4, 0.005 M MgCl₂, 0.0005 M 2-mercaptoethanol and 30% (w/v) glycerol). Remaining (NH₄)₂SO₄ was removed from the dissolved precipitate by passage through a column of Sephadex G-25, previously equilibrated with buffer (0.05 M Tris-HCl, pH 7.4, 0.001 M EDTA, 0.001 M 2-mercaptoethanol and 30% (w/v) glycerol). The flow-through fraction was applied to a phosphocellulose column (P11 Whatman), previously equilibrated with buffer. Elution was started with the equilibrating buffer containing 0.02 M NaCl, and then the concentration of NaCl was changed to 0.2 M. The 0.02–0.2 M NaCl fractions were pooled. The 60% (NH₄)₂SO₄ precipitate was collected, dissolved in the standard buffer and used as enzyme.

Table 3 Survey of primer RNA from mouse

Source of RNA	³ H-GMP incorporated (pmol)
Ehrlich ascites tumour	16.4
Spleen (normal)	1.5
(with Friend virus-induced solid tumour)	20.8
Liver (normal)	2.5
(Friend virus-infected)	15.6
(with Friend virus-induced solid tumour)	13.2
Kidney (normal)	3.2
Friend virus-induced ascites tumour	21.8
Sarcoma 180	19.0

Assay conditions were as described in the legend to Table 1. Enzyme added was 30 μg, and 20 μg of each RNA was added as a primer. RNAs were extracted from spleen and liver of mice infected with Friend leukaemia virus or transplanted with Friend virus-induced solid tumour and from Friend virus-induced ascites tumour, as described previously^{12,13}. Both the solid and ascites strains of Friend tumours were infected persistently with Friend virus¹². Mice with sarcoma 180 were supplied by Kyowa Hakko Co., Ltd.

with Friend virus-induced solid tumour and RNAs from Friend virus-induced ascites tumour and sarcoma 180 were active (Table 3). Though the biological significance of this enzyme remains obscure, these results suggest that, in mouse, primer RNAs (probably specific tRNAs) are specific to tumours or tissues infected with tumour viruses.

This work was aided in part by grants from the Scientific Research Fund of the Ministry of Education of Japan, the Waksman Foundation of Japan Inc. and the Mitsubishi Foundation.

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Received June 16; accepted July 22, 1975.

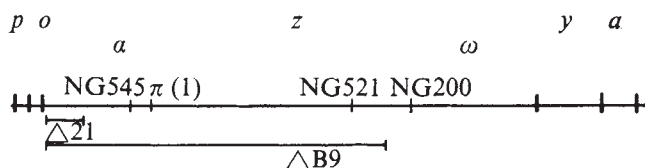
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Mutation blocking the specific degradation of reinitiation polypeptides in *E. coli*

A NONSENSE mutation in a bacterial gene leads to the synthesis of two kinds of incomplete polypeptides^{1–4}. These are the termination, or T, fragments extending from the normal N terminus to the site of mutation, and the reinitiation, or R, fragments initiating distal to the end of the T fragment and continuing to the normal C terminus. Both the T and the R fragments produced by nonsense mutations in the β-galactosidase gene of *Escherichia coli* (z gene) are rapidly and selectively degraded *in vivo*^{5–7}. This contrasts strongly with the wild-type β-galactosidase, which is stable^{5,7}. The degradation of mutant polypeptides implies the existence of proteolytic systems which can distinguish these unstable proteins from the stable ones.

One of the approaches our laboratory has taken to the study

Fig. 1 Genetic map of the *lac* operon of *E. coli*. *p*, Promoter; *o*, operator; *z*, the structural gene for β-galactosidase; *y*, the structural gene for galactoside permease; *a*, the structural gene for thiogalactoside transacetylase. The lines below indicate the extent of various deletions. Terminating mutations and π(1), the mutationally generated reinitiation site¹, are shown above the line. α and ω regions are only approximately defined by complementation. All of these mutants have been described^{9,10}. *degR* was isolated from a *recA*⁻ diploid between deletion B9 and NG200. B9 ends before the operator but covers all known *z*⁻ mutations at the operator end. This is a new observation about B9, as a result of finer mapping than in the original report¹¹.



of these degradation systems is to isolate mutations that lead to the stabilisation of T and R fragments. The first such mutation was isolated by a selective strategy based on intracistronic complementation⁸. The resulting mutation, originally named *deg* but now called *degT*, stabilises T fragments. We present results in this paper which show that *degT* mutations do not stabilise R fragments. This observation implies the existence of another degradation system which recognises R fragments. By modifying the original selection procedure we have been able to isolate a mutation, called *degR*, which stabilises R fragments against degradation but has little effect on T fragments.

To isolate mutants stabilising R fragments, a *recA*⁻ *F'* *lac* heterogenote containing two *z*⁻ mutations was constructed (see Fig. 1). These *z*⁻ mutations were picked to have such a low level of complementation that the diploid was *lac*⁻ on MacConkey *lac* indicator plates. This lack of significant complementation could be attributed to the rapid rate of degradation of the R fragment, since the T fragment was chosen for its relatively slow decay rate. The diploid was *rec*⁻ to prevent *lac*⁺ bacteria from arising by recombination. This *lac*⁻ diploid was allowed to revert, and on analysis some of the *lac*⁺ revertants were found to contain mutations affecting the degradation of the R fragment. The screening techniques were the same as previously used for *degT* (ref. 8). One of these *lac*⁺ revertants, *degR*₁, was studied extensively.

The effect of *degT* and *degR* on T fragments is shown in Fig. 2. The rate of decay of the NG521 nonsense T fragment is assayed by auto- α complementation¹². Clearly, the *degT* but not the *degR* stabilises this polypeptide. The effect of the

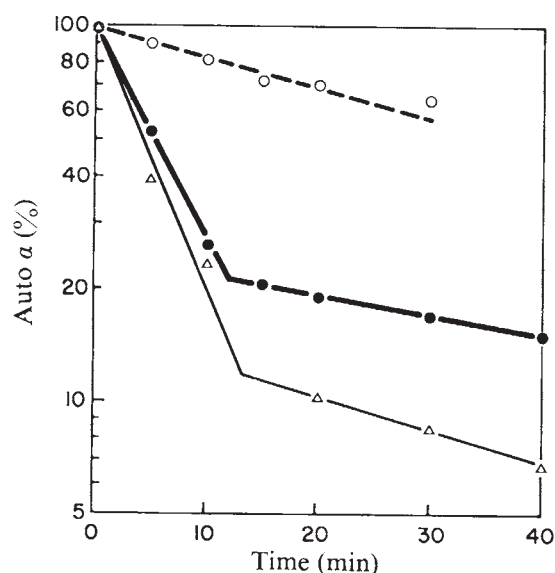


Fig. 2 Kinetics of degradation of a UGA termination fragment in *degT*, *deg*⁺, and *degR* strains. The degradation of termination fragments in exponentially growing cultures after removal of inducer was followed by assay of auto- α donor activity. The cells were grown in 100 ml of standard LB at 37 °C on a shaker, and during exponential growth IPTG (isopropyl- β -D-thiogalactopyranoside) was added to a final concentration of 5×10^{-4} M. After 15 min of induction, cells were centrifuged, washed twice with an equal volume of chilled LB, resuspended in warm LB; and the incubation was continued on a shaker. Samples (10 ml) were withdrawn at regular intervals before and after the removal of IPTG. The auto- α activity of the samples was measured as described by Morrison and Zipser¹². The β -acceptor extract was prepared from a strain carrying deletion 21 in the *z* gene. The amount of auto- α activity present immediately after the removal of IPTG was called 100%, and the percentage of auto- α activity remaining at later times was calculated accordingly. $\circ$, auto- α of 521 (UGA) fragments in *degT* cells; $\bullet$, auto- α of 521 (UGA) in wild type cells; $\triangle$, auto- α of 521 (UGA) in *degR* cells. The genotype of the strain is *F* *pro*⁺ *lac* *z*⁻ 521/*z*⁻ 521, with the exception of the *deg* marker.

two kinds of *deg* mutations on R fragments is shown in Figs 3 and 4. In one case ω complementation is used to follow decay, and in the other the ¹⁴C-labelled polypeptide is visualised by autoradiography after SDS-polyacrylamide gel electrophoresis. *degR* is seen to stabilise both the complementing activity and the physical structure of R fragments, whereas *degT* has no effect. This clear separation of the effects of *degR* and *degT* is typical. Occasionally there is some cross effect of *degR* on T fragments or *degT* on R fragments (Apte, B. N., and Zipser, D., unpublished). We believe this is not a direct effect but the result of stabilising interactions between T and R fragments themselves. Thus, for example, when a T fragment is stabilised by a *degT* mutation it can then interact with an

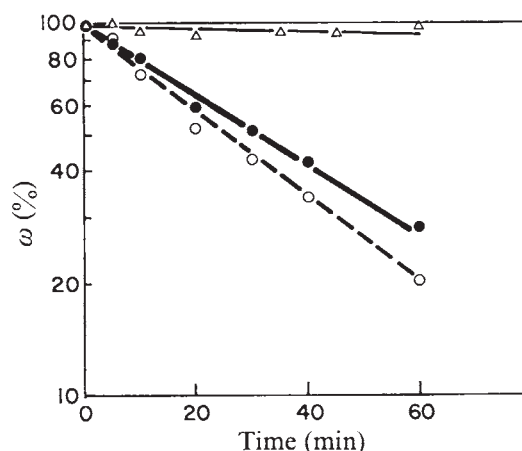


Fig. 3 Kinetics of degradation of ω complementing activity in *degT*, *deg*⁺, and *degR* strains. The cells were grown in 200 ml of standard LB at 37 °C on a shaker, and during exponential growth IPTG was added to a final concentration of 5×10^{-4} M. After 30 min of induction, cells were centrifuged, washed twice with an equal volume of chilled LB, and resuspended in warm LB; and incubation was continued on a shaker. Samples (20 ml) were withdrawn at regular intervals before and after the removal of IPTG. ω complementing activity was measured as described by Ullman *et al.*¹⁰ with some modification. The cells were collected by centrifugation; the pellet was suspended in 1 ml of TM buffer (0.01 M Tris, 0.01 M MgCl₂) and sonicated with a Biosonik IV sonicator. The sonicates were centrifuged, and 0.5 ml of the supernatant was mixed with 0.5 ml of ω acceptor extract from the *z*⁻ NG200 mutant. The mixture was incubated at 21 °C for 90 min and an aliquot was assayed for β -galactosidase activity. The amount of ω donor activity present immediately after removal of the inducer was called 100%, and the percentage of ω donor activity remaining at later times was calculated accordingly. $\circ$, ω -donor activity in *degT* cells. $\bullet$, ω donor activity in the wild type. $\triangle$, ω donor activity in *degR* cells. The genotype of the strain is *F*⁺ *pro*⁺ *lac* *z*⁻ 545- π (1)/*lac* *z*⁻ 545- π (1), with the exception of the *deg* marker.

R fragment to slow its degradation. A similar effect would be expected from stabilising R fragments.

The data in Fig. 4 indicate that the half life of the π (1) restart fragment increases from about 5 min in wild type to over 30 min in the *degR* background whereas the half life of the unstable ω complementing activity increases by at least the same factor. The apparent rate of synthesis of π (1) goes up about fourfold, as indicated by the amount of ¹⁴C-leucine incorporated in a 4 min pulse. There is also an increase of about 65% in the amount of β -galactosidase produced when a wild type *z* allele is put in the *degR* background. The cause of this modest increase is not known. It could be explained, however, if some nascent β -galactosidase was degraded by the R system in a wild type but not in the *degR* background.

The simplest interpretation of the data presented here is that *degR* is a mutation affecting some feature of the degradation system for R fragments in *E. coli*.

This investigation was supported by grants from the National

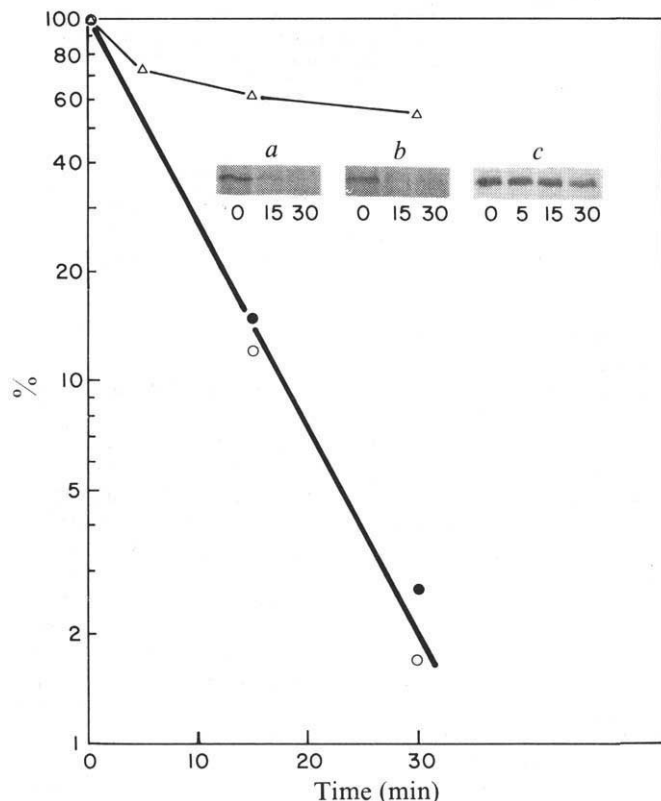


Fig. 4 Kinetics of degradation of reinitiation fragment in *degT*, *deg+*, and *degR* strains. The strains were grown in side-arm flasks containing 80 ml of minimal glycerol medium. The cells, at a density of approximately 5×10^8 cells ml^{-1} , were induced by addition of IPTG to a final concentration of 5×10^{-4} M. After 2 min of induction the culture was labelled for 5 min with 10 μCi of ^{14}C -leucine (312 μCi μmol^{-1}), at which point a 1,000-fold excess of cold leucine was added. Just before the chase and at regular intervals during the chase, 20-ml samples were withdrawn, centrifuged, resuspended in 0.5 ml of TM buffer, and sonicated. The samples were centrifuged, and 5 μg of pure β -galactosidase and 15 μl of anti- β -galactosidase serum were added to each sample. The samples were allowed to stand at 4 °C for 2 h, after which they were centrifuged and the antigen-antibody complex washed three times with TM buffer. The pellet was finally dissolved in 50 μl of SDS sample buffer by heating at 100 °C for 10 min. Fifteen microlitres of each sample were loaded on 10% SDS-polyacrylamide gel slabs, which were made and run according to Studier¹² with the discontinuous buffer system of Laemmli¹³. The gels were stained, dried, and autoradiographed on Kodak medical X-ray film. The film was scanned with a Joyce-Loebl microdensitometer. The areas under $\pi(1)$ peaks in different samples, representing the amount of radioactive $\pi(1)$ fragment present, were normalised with respect to another stable peak in all samples. The amount of fragment just before the chase was called 100%, and the percentage of radioactive fragment remaining at other times during the chase was calculated accordingly. $\circ$, Degradation of $\pi(1)$ in *degT* cells; $\bullet$, degradation of $\pi(1)$ in the wild type; $\triangle$, degradation of $\pi(1)$ in *degR* cells. A part of the autoradiogram from which the data for the decay curves were obtained is shown in the upper right corner of the figure. *a*, Degradation of $\pi(1)$ in *degT* cells; *b*, degradation of $\pi(1)$ in *deg+* cells; *c*, degradation of $\pi(1)$ in *degR* cells. The time at which the sample was taken is indicated below each sample. The genotype of the strain is $F' \text{ pro}^+ \text{ lac } z^{-545} \cdot \pi(1) / \text{lac } z^{-545} \cdot \pi(1)$, with the exception of the *deg* marker.

Science Foundation and the National Institutes of Health H.R. was a participant in the Undergraduate Research Program. We thank Don Ruthig and Sandra Lee for technical assistance.

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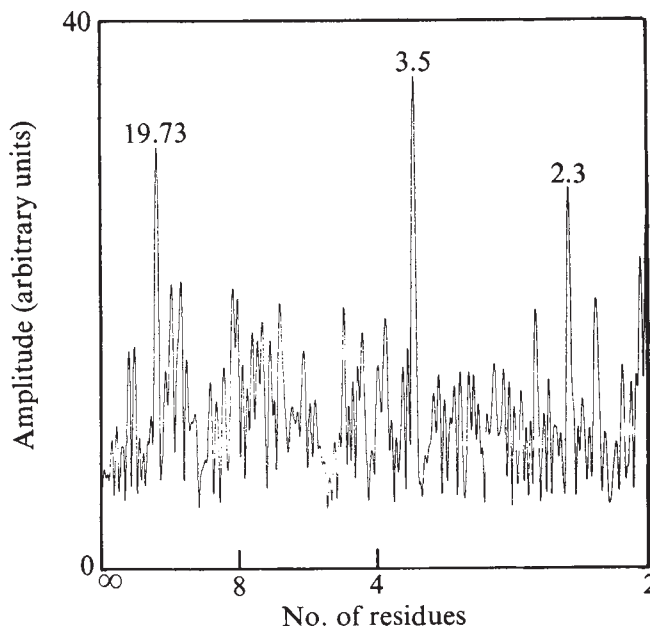
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Fourteen actin-binding sites on tropomyosin?

TROPOMYOSIN plays an important part in the control of muscle contraction. It is a rod-shaped, coiled-coil molecule, about 410 Å long, composed of two parallel α -helical chains which are in register¹⁻⁴. It lies in the grooves of the actin double helix of all known types of muscle filament and is normally thought to be associated with seven actin units⁵⁻⁷. Calcium regulates the contraction of vertebrate skeletal muscle by its influence on troponin, which in turn leads to a movement of tropomyosin in the actin groove⁸⁻¹⁰, thereby exposing (in the 'on' position) or masking (in the 'off' position) the myosin cross-bridge binding areas. The position of the troponin-binding site is known fairly precisely (ref. 11 and review ref. 4), but the actin-binding sites have not yet been identified. Here, we analyse a fourteen-fold periodicity in the amino acid sequence of α -tropomyosin¹² from rabbit skeletal muscle and propose that it is associated with seven pairs of quasi-equivalent actin-binding sites. Parry¹³ and Stone *et al.*¹² first noted several series of amino acid types with a repeat of about 19½ residues, and areas low in acidic residues spaced about 40 residues apart. There is also a slightly irregular 42-residue repeat resulting

Fig. 1 Fourier transform of the acidic residues of tropomyosin. Note the periodicity at 19.73 residues in addition to the second and third orders of the heptapeptide repeat at 3.5 and 2.3 residues. The transform was computed by expressing the chemical sequence¹² as a mathematical sequence of ones (for acidic residues) and zeros (for the rest) and, to enhance the fine detail of the transform, this was made the first 284 terms of a sequence of 2,048, the rest of which were zero. To enable different transforms to be compared directly, the mean of this sequence was adjusted to zero and its root mean square scaled to an arbitrarily chosen value of 10 before transformation.



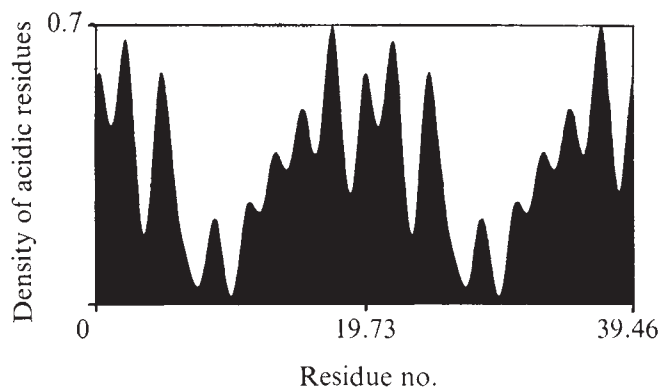


Fig. 2 Average composition of the acidic residue repeat unit (two consecutive repeats shown). The origin corresponds to the N-terminus of the sequence or points multiples of 19.73 residues from it. The distribution was computed by convoluting the mathematical sequence with a series of points spaced 19.73 residues apart. This is equivalent to adding the sequence to itself with staggers of 19.73 residues.

from gene duplication¹⁴ which is in phase with them. We used Fourier analysis to make an objective and systematic search for periodicities in the distributions of acidic, basic and non-polar groups, and here assess their significance.

Figure 1 shows the Fourier transform for the acid groups, which shows a strong periodicity at about 20 residues. By expanding the series to 17,040 terms the peak was fixed at between 19.722 and 19.745 residues, and we take 19.73 as the best estimate. The non-polar and basic distributions showed a much weaker variation 180° out of phase with the acid profile. Table 1 gives their amplitudes and phases. A detailed hand examination confirms that the periodicity persists along the entire sequence. Figure 2 illustrates the average distribution of acid groups in the 19.73-residue period and shows that they are grouped into bands about 10–12 residues wide. Thus our study shows that the periodicity is closer to 19.73 than 19½ residues, and is strongest in the negative charges. There are also marked periodicities at 7/2 and 7/3 residues, which appear because the supercoiling of the helix depends on a heptapeptide repeat pattern in the sequence^{2,12,15} of the type *a,b,c,d,e,f,g*. Residues at positions *a* and *d* are normally non-polar, at *e* often acid, and at *g* basic.

It is important to establish whether the 19.73-residue periodicity could arise by chance. To do this, artificial tropomyosin sequences were constructed by shuffling residues between heptapeptides. Residues in positions of type *a* were shuffled randomly, as were those of type *b*, and so on, producing sequences in which the heptapeptide pattern persisted but other features were randomised. These sequences were then transformed and their amplitude at a frequency of 19.73 residues determined as for the tropomyosin sequence. For the acidic residues the average value of this amplitude for 5,000 such sequences was 8.9 with a standard deviation of 4.6. The maximum value obtained was 29.4 and 99.9% of the values were less than 25.5. Therefore, the value of 31.7 obtained at this frequency for the acidic residues in the real sequence is highly significant. The low value obtained for the basic residues is clearly not significant, but the significance of the value of 20.4 obtained for the non-polar residues is not immediately clear, because the fact that these residues will only be found in the gaps left by the acidic periodicity will tend to produce a periodicity even if they occupy the gaps randomly. To test this point, random sequences were again generated, but only the positions of the basic and non-polar residues were randomised within the heptapeptide structure; the positions of the acidic residues were not changed. The mean value of the amplitude at 19.73 residues for 5,000 such sequences was 18.8 with a standard deviation of 4.3 and 27% of these values exceeded the value of 20.4 obtained with the real sequence. Thus the non-polar repeat may only be a consequence of the

Table 1 Amplitudes and phases of the transforms of the tropomyosin sequence at a frequency of 19.73 residues

	Type of residue		
	Acidic	Basic	Non-polar
Amplitude (arbitrary units)	31.7	12.0	20.4
Phase (degrees)*	13.5	168	—155

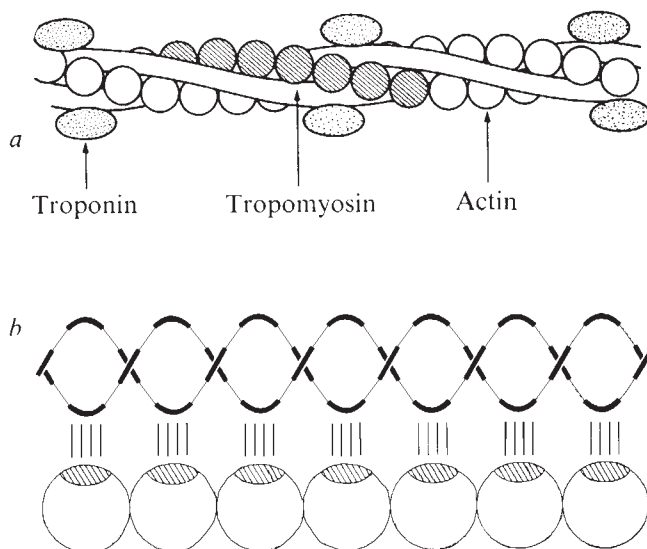
*Referred to a phase origin at the N-terminus of the sequence.

repeat in the acidic residues. A hand examination of the sequence, however, reveals some regularities in the non-polar groups at positions *b, c, f* of the helix which suggest that these groups are themselves significant.

How is the 19.73-residue length related to the structure of tropomyosin itself, and its interaction with actin? Its chains appear to be in register, so the end-to-end association of tropomyosin is thought to involve an overlap of molecular ends^{3,18}. The 19.73-residue pattern will repeat 14.39 times in 284 residues (the length of the sequence¹²) and so, if the molecules were joined without overlap, the periodicity would be interrupted on passing from one molecule to the next. Observations on paracrystals¹⁰, together with the points mentioned below about the interaction with actin suggest that there should be no interruption. This implies an overlap of about eight residues, when the apparent length of the molecule would be 276 residues, 14 times the repeat distance. If the axial residue length in tropomyosin is taken as 1.49 Å (ref. 17) a sequence of 276 residues would have a length of 411 Å, instead of 423 Å for 284 residues, which would agree well with the value of 410 ± 5 Å found in crystals⁴.

In its interaction with actin, the three-dimensional structure of tropomyosin is important. Longley¹⁸ has proposed that the tropomyosin coiled coil makes seven half turns in a molecular length. We prefer the view¹⁶, endorsed by Parry¹⁹, that there are only six half turns, so that the coiled coil makes seven half

Fig. 3 Scheme for the binding of tropomyosin to actin. *a*, Arrangement of proteins in the thin filament; *b*, interaction of seven actin units with a tropomyosin molecule. This projection corresponds to unwinding the actin helix in (*a*) so that the seven shaded actin units lie in a straight line. This operation preserves the relative positions of the actin and tropomyosin interaction sites but imparts an additional half turn to the tropomyosin coiled coil so that its apparent pitch in this projection is reduced to 114 Å as opposed to 137 Å in the thin filament. The 28 negative zones on the tropomyosin molecule (indicated on the coiled-coil by dense regions) are arranged in four series of seven and one of these series is placed so that each of its zones makes an equivalent contact with an actin unit.



turns relative to the actin subunits when it follows the twist of the actin helix in the thin filament. With the chains in register, the 14 acidic zones on each chain come together to give 28 acidic surface zones on the outside of the tropomyosin supercoil which form 14 diametrically opposed pairs 29.3 Å apart. Each pair of zones has a twist of 90° relative to the next (Fig. 3) so that the 28 zones form four series of seven when viewed along the direction of the actin helix. The zones of one of these series are spaced so that they can interact with seven consecutive actins on one side of the actin groove and it would therefore seem likely that the acid-rich zones represent the regions of the tropomyosin which bind ionically to actin, while the non-polar groups may make additional hydrophobic contacts. The 14 zones probably represent two different series of binding sites, with one set of seven being used in relaxed muscle ('off'), and one or both in the tense state ('on'), when tropomyosin changes its position⁸⁻¹⁰. The sites on adjacent zones may be so placed as to interact with both strands of the actin helix, since the projection of the 29.3 Å zone spacing also matches the axial stagger between actins. The three-dimensional reconstructions of actin-tropomyosin²⁰ seem to be consistent with these proposals.

An analysis (which will be published elsewhere) of the staining patterns in magnesium paracrystals indicates that the molecules lie with their acid-rich zones opposite one another, and suggests that magnesium bridges between opposing zones are a major source of stability in these structures. The same type of bridge would be expected to form in actin paracrystals²¹ which also appear with magnesium. As magnesium ions seem to be necessary for the binding of tropomyosin to actin (except in strong salt solutions)^{22,23} we propose that the tropomyosin-actin interaction involves magnesium bridges between zones of negative charge on both molecules, although there may also be direct salt bridges to positive zones on actin. As the charged amino acids have long flexible side chains, both types of linkage could couple residues several Å apart. This could explain the irregularities in the composition of the acidic zones of tropomyosin, since for the linkage to form it would only be necessary for the particular residue on tropomyosin to lie in a general area (probably three or four residues long).

We thank Professor L. B. Smillie for showing us his work on the sequence of tropomyosin before publication, Dr D. A. D. Parry for correspondence and our colleagues for helpful criticisms and comments. M. S. acknowledges the financial support of the Wellcome Trust and A. D. McL. the support of the Rosenstiel Foundation.

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Received June 12; accepted August 12, 1975.

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Oxygen affinity and allosteric effects of embryonic mouse haemoglobins

THE formation of a circulatory system in the ontogeny of mammals coincides with the appearance of nucleated erythroid cells which are produced in the blood islands of the yolk sac. These cells contain specific embryonic haemoglobins (Hbs) which probably transport oxygen during the earliest stages of mammalian development. Such embryonic Hbs have been demonstrated in man¹⁻⁵ and several other mammalian species⁶⁻⁸. Structural investigations of these pigments showed them to be tetramers which contain unique α -type or β -type subunits. It was also shown that the embryonic α -type subunits of man, mouse and rabbit have more sequence similarity among each other than between the embryonic and the adult α subunit of the same species, indicating that the divergence of embryonic and adult α subunits is much older than mammalian evolution⁸.

This fact is contrasted by sparse data on their function. Farooqui and Huehns⁹ have obtained oxygen dissociation curves on nucleated erythroblasts of human embryos which contained more than 30% embryonic Hbs, and found no significant difference compared with the oxygen-binding properties of human foetal red cells. Studies on the oxygen equilibrium and the Bohr effect of pure Hb Portland revealed, however, that the intrinsic oxygen affinity of this human embryonic Hb is higher than that of adult Hb¹⁰. We show here that the oxygen affinity of mouse embryonic Hbs in the presence of physiologically significant allosteric effectors is considerably higher when compared with adult mouse Hb and that Hb Portland and the embryonic mouse Hbs share some important respiratory characteristics.

Erythroid cells obtained from the peripheral blood of 12.5 d-old mouse embryos¹¹ (strain BALB/c) were washed in saline, lysed by sonification at 4 °C, and the haemolysate centrifuged to remove the cell debris. The clear supernatant was then passed over Sephadex G25 columns equilibrated with bicarbonate or bis-Tris buffer for oxygen-binding experiments in the presence and absence of CO₂, respectively. When examined by isoelectric focusing electrophoresis or ion-exchange chromatography on CM cellulose, the haemolysate contained three embryonic Hbs: Hb E_I (50%), Hb E_{II} (30%), Hb E_{III} (16%) as well as traces of adult Hb. Hb E_I consists exclusively of embryonic subunits, whereas Hb E_{II} and Hb E_{III} each contain a pair of adult α subunits^{6,8,12}. Oxygen-binding properties of the unfractionated haemolysate were examined spectrophotometrically in a diffusion chamber^{13,14} at 37 °C and the results compared with those obtained with Hb solution from adult mice (BALB/c) which had been purified by repeated passage over a mixed-bed ion-exchange resin, and then on Sephadex G25 columns.

The partial pressure of oxygen at which Hb is half-saturated (P_{50}) was lower in embryonic than in adult Hb (10.5 and 17 mm Hg, respectively; bis-Tris buffer, pH 7.2) which means that the intrinsic oxygen affinity of mouse embryonic Hb is higher than that of the adult pigment (Fig. 1), which was also found in Hb Portland¹⁰. In addition, the change in P_{50} with varying pH in the alkaline range was less pronounced in embryonic than in adult mouse Hb ($\Delta \log P_{50} / \Delta pH = -0.36$ and -0.48 , respectively). Cooperativity of oxygen binding was, however, similar in both pigments when judged from the exponent n in Hill's equation¹⁵ ($2.6 < n < 3.0$). Perhaps the most striking difference between adult and embryonic Hb with respect to the oxygen-linked proton binding is the shift of the so-called reversed Bohr effect towards neutral pH in embryonic Hb (Fig. 1). We interpret this phenomenon biologically as being advantageous for the embryo as a decrease in pH below 7.25—that is, in a severe acidosis—causes no further fall in oxygen affinity and therefore no impairment of its oxygen uptake. Oxygen release from adult Hb on the other hand, is facilitated in such a situation. The same diminished reactivity towards oxygen-linked

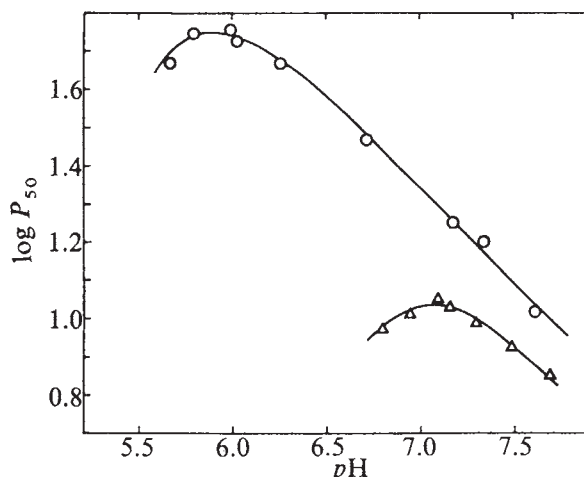
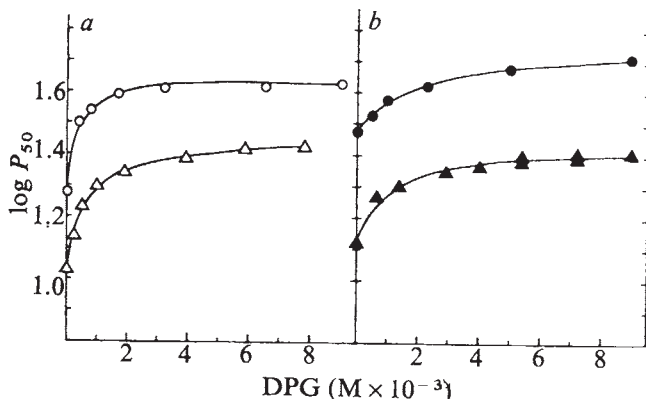


Fig. 1 Plot of $\log P_{50}$ against pH of adult (O) and embryonic (Δ) mouse Hb. Temperature, 37 °C; Hb concentration, 0.3×10^{-3} M per l tetramer; buffers, 50 mM bis-Tris-100 mM NaCl at pH ≤ 7.5 and 50 mM Tris-100 mM NaCl at pH > 7.5 . Neither preparation of adult or embryonic Hb samples contained DPG when evaluated by a kinetic test procedure¹⁸.

protons has been observed with Hb Portland, where $\Delta \log P_{50} / \Delta \text{pH}$ is only half as large as in adult human Hb¹⁰. A further similarity concerns the reversed Bohr effect which becomes effective at about pH 7 in both types of embryonic Hb and not at pH 6, as it does most mammalian Hbs including human foetal Hb.

Apart from protons, there are at least two more allosteric agents, CO_2 and 2,3-diphosphoglycerate (DPG) which influence the oxygen affinity of Hb. On addition of CO_2 ($P_{\text{CO}_2} = 40$ mm Hg) at pH 7.2, $\log P_{50}$ increased from 1.02 to 1.12 in embryonic and from 1.23 to 1.46 in adult Hb (Fig. 2). This rather weak effect of CO_2 on embryonic mouse Hb suggests that the N-terminal α -amino groups, where oxygen-linked carbamate is being formed¹⁸, are less accessible for CO_2 compared with adult Hb, possibly because the N-terminal amino groups of some subunits are blocked. The maximal effect of DPG on the oxygen affinity of adult and embryonic mouse Hb was about equal when judged from the shift in $\log P_{50}$ produced by DPG (Fig. 2a). The combined effect of both chemicals was such that at a given concentration of DPG, P_{50} for embryonic Hb was slightly lower in the presence of CO_2 than in its absence, whereas P_{50} of the adult Hb increased even further (Fig. 2b). It thus became evident that at physiological pH and temperature the high oxygen affinity of embryonic Hbs is maintained in the

Fig. 2 Plot of $\log P_{50}$ at various concentrations of DPG of adult (O, ●) and embryonic (Δ , $\blacktriangle$) mouse Hb. a, No CO_2 ; b, P_{CO_2} is 40 mm Hg. Temperature, 37 °C; pH 7.2; Hb concentration, 0.3×10^{-3} M per l tetramer; buffers, 50 mM bis-Tris and 100 mM NaCl in the absence of CO_2 (a), and bicarbonate-NaCl buffer with a molarity of 150 mEq l⁻¹ in presence of CO_2 P_{CO_2} 40 mm Hg, (b). DPG used as sodium salt.



presence of allosteric effectors which are likely to be of significance for the control of the oxygen affinity in embryonic blood.

To estimate the influence of pH, CO_2 and DPG on the oxygen affinity of Hb *in vivo*, one needs actual measurements of these variables. As far as pH and P_{CO_2} are concerned it is impossible to collect blood anaerobically from 12.5-d-old mouse embryos in quantities sufficient for the analytical procedures now available. On measuring DPG concentration, which requires less material, in embryonic blood, we found 1.35×10^{-3} M DPG per l nucleated erythroid cells compared with 10.8×10^{-3} M DPG per l adult erythrocytes. As both Hb and DPG are likely to be present only in the cytoplasm of the erythroid cells one has to correct the former value by the volume taken up by the nucleus. From a large number of measurements of the diameter of the nucleus we estimated its volume to be $250 \mu\text{m}^3$, assuming a spherical shape. Mean cellular volume of the erythroid cells was $470 \mu\text{m}^3$ so that the corrected DPG concentration is 2.9×10^{-3} M DPG l⁻¹. The mean cellular Hb concentration in the cytoplasm of these nucleated erythroid cells is 27%, giving a molar ratio of DPG to Hb tetramer of about 0.7, compared with about 2.0 in the erythrocytes of adult mice. We conclude that the concentration of DPG in erythroid cells is high enough to produce a significant decrease in oxygen affinity.

Our results show that in all experimental conditions investigated, embryonic Hb has a much higher oxygen affinity than the adult form. We conclude that the respiratory function of blood during the very early stages of mammalian ontogeny has characteristics which tend to facilitate embryonic oxygen uptake.

More structural and functional analyses of embryonic Hbs should provide an answer to the question of whether the high oxygen affinity of these respiratory proteins is a selective factor in evolution, as seems to be the case for the salient allosteric effects of adult Hb¹⁷. In favour of such an assumption is the similarity of intrinsic oxygen affinity and Bohr effect of mouse and human embryonic Hbs.

This work was supported by the Deutsche Forschungsgemeinschaft.

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Conformation of thyroid hormone analogues

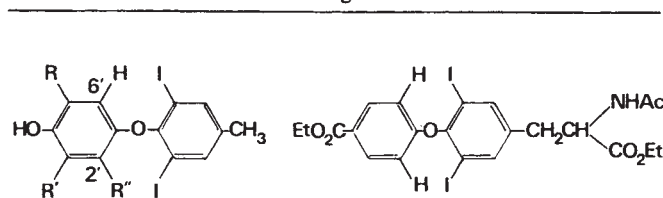
COMPARISON of the activities of conformationally 'fixed' derivatives of thyroid hormones has led to the proposal that the active conformation of 3,5,3'-triiodo-L-thyronine (L-T₃) is one in which the 3'-iodine is distal to the inner (α) ring¹⁻⁴ (Fig. 1). These conclusions have prompted several X-ray studies aimed at determining the conformational preference in the crystals of thyroid hormones⁵⁻¹⁰. Such studies have shown that the posi-

tioning of the 3'-iodine in the crystal structures of L-T₃ and related compounds is dependent on the crystallising medium, thus suggesting that the distal and proximal forms of L-T₃ are of similar energy. These findings prompted us to investigate the temperature dependence of the proton nuclear magnetic resonance (NMR) spectra of thyroid hormone analogues to determine, in solution, both the magnitude of the barrier to rotation around the ether oxygen and, in unsymmetrical molecules, the relative populations of distal and proximal forms.

Previous work¹¹ on 2,6-diisopropylidiphenyl ethers, together with reported steric parameters for iodine and isopropyl¹², suggested that the coalescence temperature for the 2',6' proton signals of thyroid hormones would be below -60 °C. Since, at this temperature, the thyroid hormones would be practically insoluble in most solvents, we chose, instead, to investigate the model compounds (1-4 in Table 1). The steric and electronic consequences of replacing the alanine side chain by a methyl group are not expected to affect markedly the intrinsic rotational properties of the ether bond⁴. Nevertheless, we recognise that this may alter the dipole moment of the molecule and that this in turn may affect the conformer population in solvents of high dielectric constant¹³.

The 60-MHz spectrum of the thyroxine model compound (1) (Table 1), in tetrahydrofuran, shows a time averaged singlet (7.18 p.p.m.) for the 2',6' protons at 40 °C. On cooling, this

Table 1 Coalescence of 2',6' protons of thyroid hormone analogues



- (1) R = R' = I, R'' = H
 (2) R = H, R' = I, R'' = H
 (3) R = R' = R'' = H
 (4) R = R' = H, R'' = Me

(5)

Compound	T_c (°C)	$\Delta\nu$ (Hz)	$\Delta G_c^\ddagger$ (kcalorie mol ⁻¹)*
(1)	-94 ± 3	76.5	8.5 ± 0.2
(2)	-105 ± 4	73 (2'-H) 68 (6'-H)	7.9 ± 0.3
(3)	< -110 ± 2	—	< 7.7
(5)	-79 ± 3	65	9.3 ± 0.2

* 4.18 J = 1 calorie

Spectra were recorded on a Varian A60A (60 MHz) spectrometer in sodium dried tetrahydrofuran. Estimated errors in ΔG were based almost exclusively on uncertainties in T_c .

singlet broadens and eventually separates into two broad peaks (6.57 and 7.84 p.p.m.) (Fig. 2). At the coalescence temperature (-94 °C) a rotational barrier ($\Delta G_{179}^\ddagger$) of 8.5 ± 0.2 kcalorie mol⁻¹ was calculated (Table 1) from the Eyring Equation¹⁴.

At 40 °C, the spectrum of a solution of the T₃ model compound (2) in tetrahydrofuran shows a typical ABC pattern for

Fig. 1 Distal (a) and proximal (b) conformations of T₃ analogues.

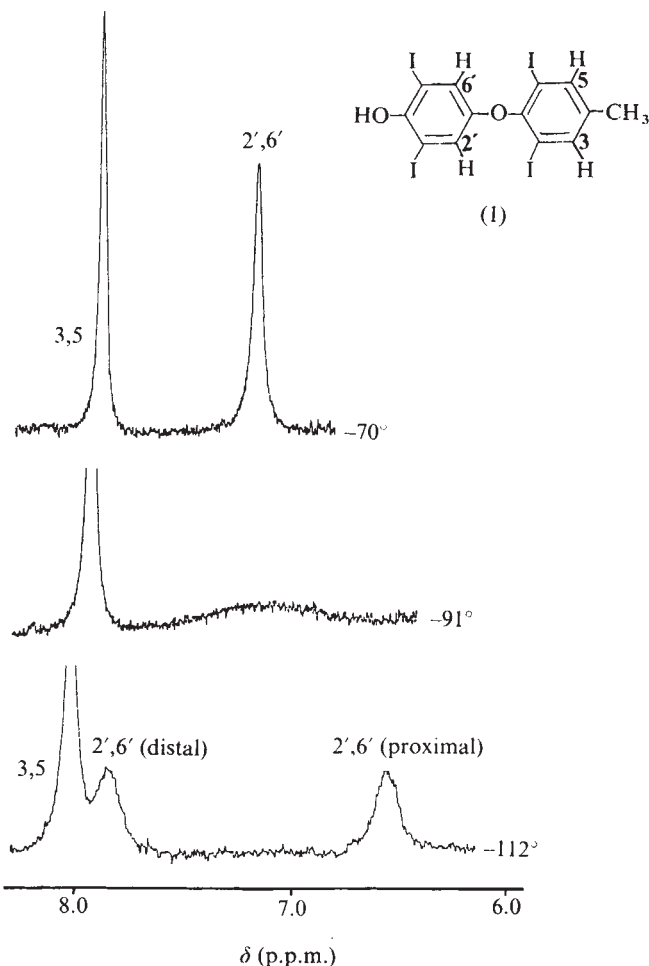
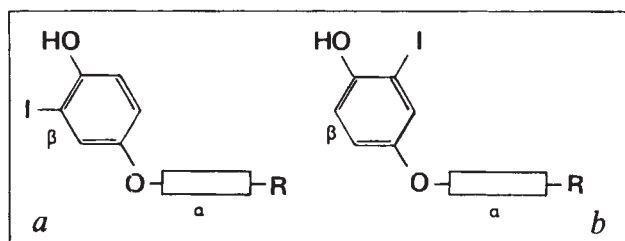


Fig. 2 Variable temperature NMR spectra of compound (1).

the 2', 5' and 6' protons (Fig. 3). The spectrum was analysed with the aid of a simple ABC computer program which provided accurate chemical shifts¹⁵. On cooling the solution to -108 °C, at which temperature rotation about the C(1)-O bond was slow on the NMR time scale, we expected to see the original ABC pattern replaced by two overlapping ABC patterns. Only signals for the individual 2'-protons in each of these two ABC patterns can be easily distinguished, however. Thus the 2' proton (7.1 p.p.m.) separates into two broad single peaks (6.5 and 7.72 p.p.m.) of equal intensity (Fig. 3). At the coalescence temperature (-105 °C) a rotational barrier ($\Delta G_{168}^\ddagger$) of 7.9 ± 0.3 kcalorie mol⁻¹ was calculated (Table 1). At -108 °C the 6' proton separates into two broad peaks with approximate shifts of 6.1 and 7.3 p.p.m. (Fig. 3). The T₂ derivative (3) showed only exchange broadening of the 2',6' proton signals at the solvent limit of -110 °C, indicative of a barrier to rotation ($\Delta G_{163}^\ddagger$) of less than 7.7 kcalorie mol⁻¹.

It is apparent from these results that rotation around the ether bond is governed by non-bonding interactions between the 2',6' hydrogens and 2,6 iodines. If it is assumed that the entropies of activation ($\Delta S^\ddagger$) for rotations in compounds (1) and (3) are small^{14,16,17} or similar¹⁸ then comparison of $\Delta G_c^\ddagger$ (Table 1) for these two compounds suggests a slightly larger value for the T₄ derivative (1). This difference, if real, is probably caused by the buttressing effect of the 3',5' iodines on the 2',6' hydrogens, an effect previously observed in substituted biphenyls¹⁸. An indication that electronic factors may also affect rotation, as in other systems¹⁷, is suggested by the higher barrier to rotation ($\Delta G_{194}^\ddagger = 9.3 \pm 0.2$ kcalorie mol⁻¹) found for the diester (5, Table 1) compared with that of the phenol (3), assuming $\Delta S^\ddagger$ is similar for these two derivatives.

The 2' proton signals in the low temperature (-108 °C)

spectrum of the T_3 model compound (2) were of approximately equal intensity (Fig. 3), indicating an almost equal population of distal and proximal conformations. This represents the first experimental evidence indicating the existence of two conformations of T_3 or one of its analogues in solution. In extrapolating these low temperature results to biological temperature, it was necessary first to determine the variation of the weighted average chemical shifts of the 2' and 6' protons of (2) with temperature in the absence of changes in conformer population. Graphical plots showed that all protons in compounds (1–4) suffered chemical shift changes due to the influence of a common solvent effect. The results for compounds (3) and (4) are of particular interest since they involve only one conformation. The chemical shift of the proximally positioned¹⁹, 6' proton of the 2'-methyl derivative (4) showed a shift towards low field of only 5 Hz over the range +40 to -110 °C. The average chemical shift of the 2' and 6'-protons of (3) showed a similar change. We can conclude from these results that both proximal (2',6') and distal (2',6') protons show conformationally independent chemical shift changes with temperature, but these changes are likely to be (a) small compared with the chemical shift difference between 2' (or 6') proximal and distal protons and (b) similar for proximal and distal protons, that is, the chemical shift difference ($\Delta\nu$) is largely temperature independent. These results enable us to calculate distal-proximal ratios at different temperatures from the equation

$$n_D = \frac{(v_{W2'H} - v_{W6'H}) + (v_{D6'H} - v_{P2'H})}{(v_{D6'H} - v_{P6'H}) + (v_{D2'H} - v_{P2'H})} \quad (1)$$

where n_D = mole fraction of distal form, and v_D , v_P and v_W refer respectively to distal, proximal and weighted average

chemical shifts. v_D and v_P were obtained from the single spectrum of (2) at -108 °C (Fig. 3) and n_D is calculated at any higher temperature by measuring $v_{W2'H} - v_{W6'H}$ at that temperature. On substituting $v_{W2'H} = 427.3$, $v_{W6'H} = 393.7$, $v_{D6'H} = 435$, $v_{P6'H} = 367$, $v_{D2'H} = 463$ and $v_{P2'H} = 390$ Hz into the above equation, n_D (40 °C) is calculated to be 0.56 (range 0.5–0.6 after taking account of experimental error); this indicates that there is very little change in the conformer ratio over the temperature range studied.

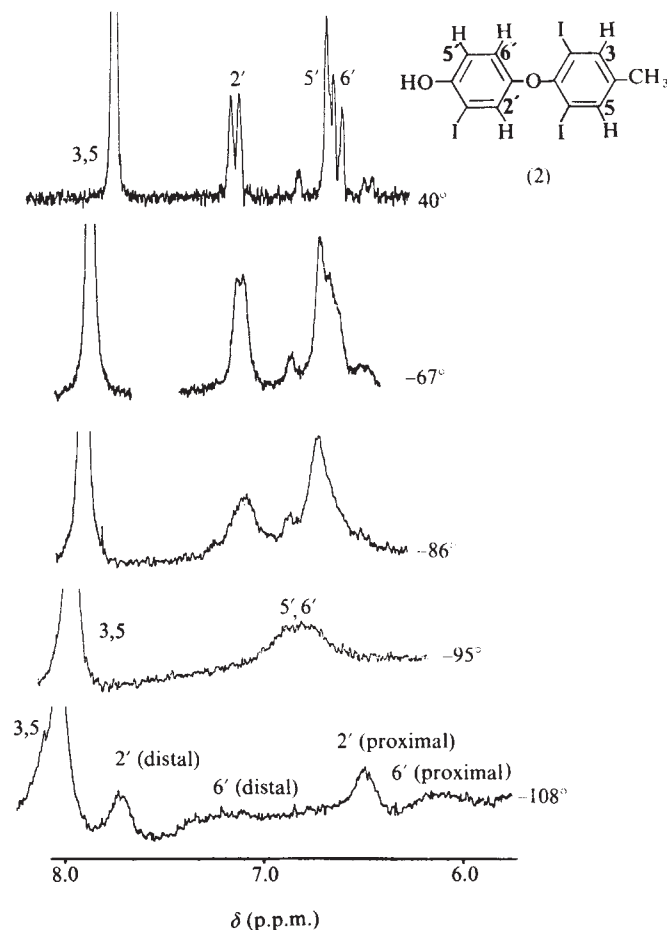
In summary, these results indicate that the barrier to rotation around the ether oxygen in thyroid hormones is in the range 7–9 kcalorie mol⁻¹, in reasonable agreement with a calculated⁴ barrier (11 kcalorie mol⁻¹) for T_3 . Furthermore, the observation of approximately equal populations of distal and proximal forms of the T_3 analogue (2) is not incompatible with an 'active' distal form, as suggested previously^{2,3}.

A recent communication by Camerman *et al.*²⁰ reported the observation of a single species of 3,5,3'-triiodothyropropionic acid in a mixture of diethanolamine, urea and methanol, but claimed to have shown the presence of two non-interconvertible conformations in a 2:1 mixture of ethanol-1 N HCl at room temperature. We believe that this work is in error as, first, the reported shift difference (1.7 Hz) between the 2' proximal and distal protons is negligible compared with those observed by us and other workers¹¹ and, second, the barrier to rotation, reported to be in excess of 20 kcalorie mol⁻¹, is far higher than expected. These apparently anomalous results are readily explained, however, if one assumes partial esterification of the acid by the acidic ethanol used as solvent.

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Fig. 3 Variable temperature NMR spectra of compound (2).



Received April 28; accepted August 13, 1975.

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Errata

In the article "Eccentricity-specific dissociation of visual functions in patients with lesions of the central visual pathways" by E. Pöppel *et al.* (*Nature*, **256**, 489; 1975) Fig. 1 and its legend should have been deleted and do not refer to the paper as published.

The title of the article (*Nature*, **256**, 750; 1975) by B. Donzel *et al.* should have read "Synthesis and conformations of hypothalamic hormone releasing factors: two TRF-analogues containing backbone N-methyl groups" and not as printed.

matters arising

February–June weather relationships in Norway

GREEN¹ claims that the empirical prediction technique can be useful in the development of long range weather forecasting. Empirical methods of long range forecasting sometimes follow the discovery of relationships between observations made at different times and places. Though I agree with Green that there seems to be no reason not to use the relationships in prediction, even though the physical mechanism behind them is not fully understood, some precautions must, nonetheless, be taken. If empirical correlations are to be of any value in weather forecasting, correlation coefficients ought to be considerably larger than the significance levels.

Green illustrated his idea using as an example the correlation between February and June temperatures at Dalen in Norway, between 1940 and 1974. He claims that a cold February will be followed by a warm June. Figure 1, however, shows that points are quite scattered.

An analysis of observed winter and summer temperatures in Scandinavia indicates that no correlation exists that can be useful for a forecast model. Fairbridge² has given differences between July and preceding January temperatures in Stockholm from about 1750. The data indicate a decline in amplitude during the nineteenth century, but a marked increase in recent years.

Table 1 shows the results of a correlation analysis of monthly temperatures at Dalen in Norway. The significance limit is based on a confidence

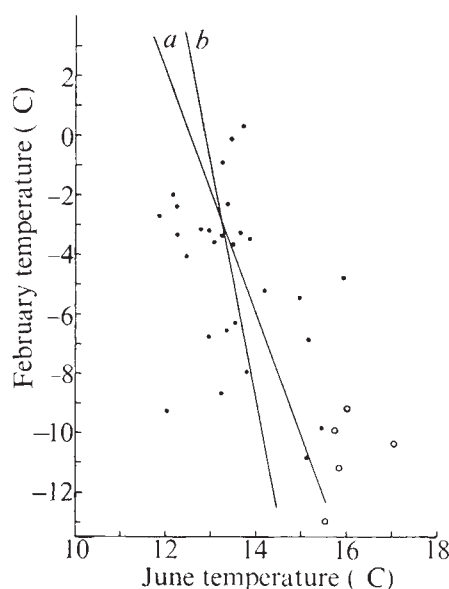


Fig. 1 Relationship between February and June temperatures at Dalen, Norway between 1940 and 1972. *a*, Computed from February–June temperatures for the period 1940–72; *b*, based on the same temperatures except those indicated by circles, showing that the negative correlation depends strongly on a few extreme temperature conditions.

level equal to 95% in the Student's *t* distribution, and the coefficient is given only when it exceeds the significance limit. For the complete series, 1890–1972, there is no significant correlation between the February–June temperatures. Only by considering a shorter period back from 1972, can a significant correlation be found. Nor is the correlation between the lowest and the highest monthly temperature in the year significant. Similar results have been found in Oslo and Hellisøy.

Table 1 Empirical correlation coefficients for monthly temperatures at Dalen, Norway, between 1890 and 1972

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
January	1.00	0.52	+	+	—	—	+	+	+	+	—	—
February		1.00	0.30	0.30	—	—	+	—	+	+	+	—
March			1.00	0.48	+	+	+	—	+	+	+	+
April				1.00	0.39	—	+	+	+	+	—	+
May					1.00	+	+	+	+	—	—	+
June						1.00	+	+	0.23	+	—	+
July							1.00	0.42	0.22	+	+	—
August								1.00	0.36	+	+	+
September									1.00	0.29	+	—
October										1.00	+	—
November											1.00	0.37
December												1.00

* Significance limit r_s 0.22.

In Britain³ and Norway, the monthly rainfall and temperature are correlated positively in winter and negatively in summer. If a negative correlation between February and June temperatures exists, a positive correlation between February temperature and June rainfall can be expected. An analysis of June rainfall and the temperature of the preceding months for three different places, Oslo, Dalen and Bergen reveals no significant correlation except for February temperatures in Bergen, in which the empirical correlation coefficient is just on the significance level.

I conclude that there is no statistical evidence that the winter temperature is a useful element in forecasting the summer temperature and rainfall for the same domain.

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¹ Green, F. H. W., *Nature*, **253**, 522–523 (1975).

² Fairbridge, R. W., *Encyclopedia of Atmospheric Sciences and Astrogeology*, 205–211 (1967).

³ Murray, R., *Met. Mag., Lond.*, **96**, 141–145 (1968).

Development of visual acuity and the sensitive period

WE have read with interest the article, by Freeman and Marg¹. Their observations that there is a significant change in the response of the visual system of the kitten to spatial gratings with age are not unexpected from the results of our study of the optical properties of kitten's eyes. We question, however, their contention that, on the basis of their ophthalmoscopic observations, "it is unlikely that optical factors limit the acuity determined for younger animals." As evidence to the contrary, we submit the following.

Photographs of the fundus of the same kitten 11, 18, 25 and 32 d after birth are shown in Fig. 1. These photographs were made with a Zeiss fundus camera and accurately demonstrate the hazy media one encounters in kittens for the first 5 weeks of life. This haziness results from the scattering of light by the cornea which is not optically clear in the first 3–4 weeks of life, the tunica vasculosa lentis and by particulate material in the vitreous, much as is seen in premature infants (A. McCormack, personal communication). The tunica vasculosa lentis disappears between days 30 and 35 of life and not before day 23 as contended by Freeman and Marg. To quantify the

degradation of an image by the media of the eye, we have made densitometric measurements of a fluorescein-filled vessel in the posterior pole of the eye. Between days 25 and 32, there was a 43% decrease in the spread of the image of the vessel in the absence of any change in the refractive power or the axial length of the eye. These observations demonstrate that the optical quality of the kitten's eye is improving over this period and must play a significant part in determining the acuity of kittens.

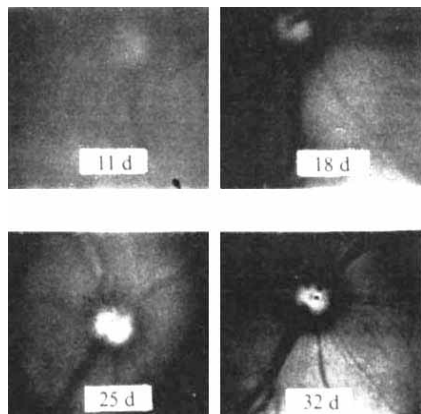


Fig. 1

Further, there is another important factor about the kitten's eye which must be considered by the visual physiologist. We have determined, by retinoscopy, the refractive error of eight eyes from seven kittens. During the first eight weeks of life, with the accommodation paralysed by atropine, the mean refractive error is 7.62 D (range +15.00 D to +4.00 D). It should be noted that these are refractive error determinations for an object at infinity, and any object placed closer will require the kitten to use its accommodative power, which may be limited by the anterior displacement of the lens and its envelopment by the tunica vasculosa lentis. Thus, for example, if the kitten with a 7.50 D hyperopia were placed in the middle of a 50-cm diameter striped cylinder, it would be required to employ 11.5 D of accommodation to bring the stripes into focus. For the reasons cited above, we do not believe the kitten possesses anywhere near this accommodative power. It is indeed surprising that, in spite of these optical defects, it is possible to alter the development of receptive field properties of cortical cells², although these results have not been confirmed (M. P. Stryker and H. Sherk, unpublished). For an out-of-focus optical system with a vertical slit pupil, vertical objects will be in better focus than horizontal objects. If the kitten's visual system responds as does the human astigmatic eye³, the question arises, why are there not more vertically tuned cortical cells in normally

reared cats?

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¹ Freeman, R. D., and Marg, E., *Nature*, **254**, 614 (1975).

² Blakemore, C., and Cooper, G. F., *Nature*, **288**, 477-478 (1970).

³ Mitchell, D. E., Freeman, R. D., Millodot, M., and Hagerstrom, G., *Vision Res.*, **13**, 535-558 (1973).

FREEMAN AND MARG REPLY—Accommodation and refractive error has nothing to do with our study as has been suggested¹. The kittens were under general anaesthesia and the eye and its protective contact lens were optically corrected for the 57-cm screen distance.

Optics has a great deal to do with the development of kittens and the determination of their sensitive or critical period of development. It is by means of slits, bars, and edges that the concepts of visual development and deprivation have been uncovered. The original work of Hubel and Wiesel² demonstrated orientation of cortical receptive fields in the kitten as young as 8 d, and others have done similar experiments. The optics of these eyes must be transmitting those forms.

There is no question that the optics of the kitten's eye improves up to about the fifth week, but this does not mean that it is the limiting factor in acuity. With good illumination (rather than a fluorescent source) retinal vessels of about 0.5° width can be resolved at 3 weeks of age. It is likely that the optics are transmitting to the retina gratings of similar detail.

While we await the results of systematic optical modulation transfer function data from the kitten, we stand by our original statement:

"It is clear that the optical quality of the eye of the very young kitten is inferior to that of the adult. But on the basis of ophthalmoscopic observation we feel that it is unlikely that optical factors limit the acuity determined for the younger animals."

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¹ Flynn, J. T., Hamasaki, D. I., Flynn, T. E., and Barricks, M., *Nature*, **257**, 337-338 (1975).

² Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **26**, 994-1002 (1963).

Basalt from DSDP holes

ALTHOUGH Hammond *et al.*¹ are concerned mainly with palaeolatitudes of the Ontong Java Plateau, where DSDP 289 was drilled, they give considerable attention to the basalt at the bottom of the hole. At long last it is good to see another radiometric age for basalt, no matter how flawed the age may seem to the authors. The latter "assign" an age of 109 Myr to

the basalt on the assumption that it must be older than sediments above it which are dated as 108 Myr on biostratigraphic grounds. They find that the radiometric age of 80 Myr which they actually obtain is in "error", and they cite a similar error in a radiometric analysis performed by Cox and Dalrymple² to conclude that the "estimated analytical standard deviation arises almost entirely from uncertainties in the potassium measurements".

I can only suggest that uncertainties cut both ways. If a basalt dated at 80 Myr is to be corrected to 109 Myr it could just as well be corrected in the opposing direction to 51 Myr. In the latter case the basalt would be a sill. Needless to say the basalt would also be a sill if the authors were willing to accept their own date. As to their citation of Dalrymple and Cox I myself would cite MacDougall³. He found a basalt below Campanian strata in DSDP 10, western Atlantic, that gave a radiometric age of 16 Myr. The result was acceptable to MacDougall, from which one concludes that the basalt of DSDP 10 is a Miocene sill intruded into Campanian strata.

Those are the facts; however, impressions may not be out of place. The authors give me the impression that they are "bound and determined" to have the basalt as the oldest material in DSDP Hole 289. Why? Almost certainly because they assume that the basalt is basement. That is a common assumption in modern tectonophysics, but in my opinion it needs to be refuted vigorously. The fact that a few fragments of basalt are the oldest material in a particular hole has no connection with its crustal nature. If the basalt in DSDP were 218 Myr old instead of the assessed 109 Myr old there could still be 1,000 m of little-disturbed strata below. In short, the age of the ocean basins is a problem as wide open now as it was when DSDP drilling began.

In the last analysis my observations are addressed not so much to Hammond *et al.* in particular as to shipboard scientists in general. The scientists seem to go to sea convinced and/or indoctrinated that every piece of basalt is not only the bottom of the stratal sequence but also the bottom of the world. I have no proof to offer that their conviction and/or their indoctrination is incorrect. I submit only that if we are to be comparatively scientific about the nature of the ocean basins we had better drill some 20,000 m of basalts in suitably distributed DSDP holes. Only then could there be a geological dialogue of substance with respect to some of the matters indicated here.

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¹ Hammond, S. R., *et al.*, *Nature*, **255**, 46 (1975).

² Cox, A., and Dalrymple, G. B., *J. geophys. Res.*, **72**, 2603 (1967).

³ MacDougall, D., *Science*, **171**, 3977 (1971).

reviews

Waves or particles?

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THE discovery of X rays by Röntgen in 1895 gave rise to considerable speculation as to whether this radiation was corpuscular, like electrons, or undulatory, like light. As the experimental evidence grew so it became apparent that, like light, X rays must be a form of electromagnetic radiation; Barkla demonstrated that X rays could be polarised and in accordance with J. J. Thomson's theory, this was taken as clear evidence for their being electromagnetic waves; this conclusion was reinforced some years later by Friedrich, Knipping and Laue, who showed that they could be diffracted. Nevertheless there was one property of light which eluded a satisfactory explanation on this wave basis and that was the photoelectric effect. The only simple explanation for this phenomenon was given in 1905 by Einstein, who published his well-known paper on the light quantum interpretation of the photoelectric effect and derived his famous relation $eV = h\nu$. Not much notice was taken of Einstein's contribution and the view was firmly held by the majority of physicists that light and X rays were electromagnetic waves and this therefore excluded them from possessing particle properties. Even as late as 1916 Millikan, in the paper reporting his experiments on the photoelectric effect which exactly confirmed the Einstein relation, commented: "Yet the semicorpuscular theory by which Einstein arrived at his equation seems at present to be wholly untenable". This relationship was accepted as convenient and reliable and it was presumed that it would ultimately prove explicable on electromagnetic theory.

It was at this stage that A. H. Compton came on to the scene. He had started his research under O. W. Richardson at Princeton in 1914 on X-ray diffraction and, after some abortive experiments to look for magnetic scattering of X rays, commenced his experiments on the spectrum of the scattered radiation. These were absorption experiments and they showed that, together with the radiation of unaltered wavelength, there also seemed to be softer radiation present. Barkla had observed this radiation

and interpreted it as fluorescent radiation characteristic of the scatterer—but longer in wavelength than those lines which can be understood by the Bohr model—and named it J radiation. Compton, adhering strongly to electromagnetic theory, chose to interpret the changed adsorption coefficient not as a change in wavelength, but as being due to the size and shape of the electron: by assuming that it was a rather large object and of ring shape, he found that he could account for the reduced absorption coefficient in the conditions of his experiment. After visiting Cambridge in 1919, where Rutherford viewed his large electron with some scepticism, he returned to the US and attacked the scattering problem again, this time using a Bragg spectrometer as a monochromator of the incident X rays. He then found an explanation for the softening of the X rays in terms of Doppler displacement of the radiation from the moving electron, which had received momentum from the incident X rays. He was still, however, unable to account satisfactorily for all the features of scattering and it was only at this stage in October 1922 that he found that a corpuscular interpretation would account fully for all his observations. Quite quickly, most physicists accepted this interpretation of

his convincing experiments and thus accepted the particle nature of electromagnetic radiation. Thus, some 17 years after Einstein's original paper on the photoelectric effect, the scientific community recognised the fact that light and X rays each possessed both wave and particle characteristics.

In this book* Dr Stuewer, an historian of science, traces the ideas and background to Compton's experiments which were to prove important in the development of wave-mechanics. The author, using material drawn from published papers and letters, guides the reader through much of the history of the particle-wave duality. It is liberally referenced and sprinkled with detailed theoretical arguments, and the tenacity with which physicists held to the classical electromagnetic theory emerges very clearly in this book. This piece of physics history will be primarily of interest to physics graduates and, although lacking the gossipy and humorous touches of biography, it nevertheless provides a vivid, enjoyable and definitive account of one of the crucial steps in the establishment of quantum mechanics. It is an unusual work and should have a place in the library of the physicist who is interested in more than the bare bones of his subject.

M. A. Grace

**The Compton Effect: Turning point in Physics*. By Roger H. Stuewer. Pp. xii+367. (Science History: New York, April 1975.) n.p.

Biochemical and biophysical aspects of photosynthesis

Bioenergetics of Photosynthesis. (Cell Biology: A Series of Monographs.) Edited by Govindjee. Pp. xiv+698. (Academic: New York and London, April 1975.) £20.65.

OFTEN books born of long gestation periods are disappointing when they finally arrive. That is definitely not the case with this well edited volume, which should have a very useful life in the library and on the desk.

The book comprises 12 chapters by selected authors who cover the general biochemical and biophysical aspects of photosynthesis in plant and (to a lesser extent) bacterial systems. The way in which this is related to bioenergetic conversion is dealt with from several points of view: membrane structure related to pigments and energy conversion; chloroplast structure; the primary events of photosynthesis including luminescence and fluorescence; oxygen evolution; electron transport; phosphorylation; and ion movements. Each chapter has been externally reviewed and the editor has inserted useful cross references. This, combined with the extensive references, the list of abbreviations and the tables, ensure that each chapter, though complete in itself, is also relevant to other chapters.

The subject index and author index are excellent—necessarily so in a book which claims to “serve as a reference source for researchers but also as an introductory work for graduate students”—and, consequently, the book is more useful than volumes of proceedings and monographs which lack such indexes. It is a pity that more publishers do not insist on the inclusion of extensive indexes even though the resulting books may be more expensive than otherwise—in the long run readers would find the extra expense well worthwhile.

Since the practical use of photosynthesis based on both the improvement of its natural efficiency and the construction of artificial systems are so in vogue now (for food and/or energy) it is a pity that a chapter on this important topic was not included.

An understanding of the biochemical and biophysical mechanisms of photosynthesis seems crucial to its future exploitation and this is where this book will provide the source of background information for present and future investigations—whether scientific, administrative or commercial. **D. O. Hall**

Magnetic oxides

Magnetic Oxides. Edited by D. J. Craik. Part 1: Pp. xxi+482. Part 2: Pp. xix+483–798. (Wiley: London and New York, 1975.) £15.00 each.

THESE two volumes cover various aspects of the theory, properties and uses of magnetic oxides. The 13 chapters are written by 20 authors and cover an extremely wide range, from the practical arts of crystal growing to the theoretical intricacies of crystal fields and exchange. The versatility of magnetic oxides as recording media is also well covered in chapters ranging from the recently utilised bubble domains to the recording over geological times of past terrestrial magnetic fields in the oxides which occur naturally in rocks. There are also chapters on such topics as anisotropy, magnetostriction, optical properties, electrical properties, domains and microwave resonance. Experimental techniques such as neutron diffraction and nuclear magnetic resonance are, of necessity, mentioned here and there but are not discussed fully.

The question of units in a multiple

author book on magnetism is bound to present an initial problem, at least, and it has been solved in this case using e.m.u. throughout, in line with most of the literature. It is, perhaps, not surprising in view of the wide field that the treatment of many of the topics is not detailed enough to be followed by the reader without frequent recourse to references, and in that respect the book tends to be a rather thorough review of the current state of knowledge. That is, however, the major strength of the work and each chapter is well provided with a comprehensive list of references, there being something like 1,700 in the whole book.

Although the individual chapters are to a large extent independent of each other, the work as a whole is well integrated by frequent cross-referencing where interaction of subject matter does occur. The book is perhaps most likely to appeal to the specialist who wishes to extend his knowledge of other aspects of magnetic oxides, and for that purpose it is of considerable value in view of its logical presentation and comprehensive referencing. **A. Stephenson**

Kets, bras and boson variables

Spinors in Hilbert Space. By P. A. M. Dirac. Pp. vi+91. (Plenum: New York and London, 1974.) \$14.50.

ON reading this volume, I had at once an impression of *déjà vu*, hearing again the logical, elegant and irresistible exposition so well known to us from Professor Dirac's classic work *The Principles of Quantum Mechanics*. This book differs from the latter, however, in being primarily mathematical in character. Its relevance to the physics of the real world is mentioned in only a few of its 35 sections. The book is based on a series of lectures which the author gave at the University of Miami in 1969. Although self-contained, it is not really intended for the beginner; for example, familiarity with the notion of a spinor and its properties and uses is very much taken for granted, although it is true that four lines in Section 2 do give a complete characterisation for a spinor quantity.

Dirac begins by developing the theory of vectors and operators in a real Hilbert space with $2N$ dimensions, in which there exists the concept of perpendicularity (as opposed to orthogonality, which concept also exists). He then introduces the operation of rotation and distinguishes spinors from tensors by the reversal of sign experienced by the former for a complete rotation. In this space, the vectors, termed kets, and their duals, termed bras, are the most elementary spinors, and Dirac confines his attention to them, since more complicated spinors can be constructed from them by multi-

plications, as done by van der Waerden in his lectures on spinors some decades ago. Their possible application to physical problems is not discussed in detail here, although Dirac does comment briefly about this at several points in the lectures, identifying, for example, the operators for the creation and destruction of fermions.

Dirac then approaches the case of special interest for him, the case of a Hilbert space with an infinite number of dimensions, by taking the limit $N \rightarrow \infty$. For this, the operators of interest are those whose matrix is bounded, so that the operations of multiplication and division are defined for them. This limit leads Dirac to a new class of operators, which do not commute with each other nor obey the associative law of multiplication. He terms these as ‘boson variables’ and they come in two types, identified by Dirac as creation and destruction operators. In the closing section, Dirac emphasises that these boson variables appear automatically in infinite-dimensional theories which start with only fermion variables, provided that the latter are infinite in number. Dirac observes, “There must be such boson operators connected with electrons. Their physical presence is a subject for further investigation. They have a negative energy...”

This book is very much for the specialist, and one may expect it to have much value and influence in suggesting new extensions from the physical theories which we now know. We must note, however, that this little book costs 15 cents per page, which, even given the circumstances, does seem unnecessarily high.

R. H. Dalitz

Group theory and electronic states

Symmetry of Many-Electron Systems. (Physical Chemistry: A Series of Monographs, vol. 34.) By I. G. Kaplan. Translated by J. Gerratt. Pp. xii+370. (Academic: New York and London, February 1975.) \$34.50; £16.55.

GROUP theory has been used for many years in the quantum mechanical analysis of many particle systems. This monograph deals only with applications to many electron systems, specifically atoms and molecules, the physical properties of which reflect both the symmetry with respect to the permutation of the identical particles of which they are composed and the symmetry of the potential field formed by the nuclei within which these particles move. Other fields of application are ignored.

The book opens with an account of some of the main facts of group representation theory presented as a tool for the chemist, which omits many of the proofs and derivations. The permutation group is singled out for detailed study, an understanding of Young tableaux and Young operators being essential in later chapters. Linear groups of transformations (including the three-dimensional rotation group), point symmetry groups,

and the construction and uses of tensor representations and irreducible tensor operators are all described briefly.

The rest of the book is devoted mainly to the classification and calculation of electronic states of atoms and molecules. Considerable use is made of fractional parentage expansions, which are worked out in detail for the atomic case in $j-j$ coupling and then generalised to handle the non-vector-coupled states that are of importance in molecules. They allow one to construct matrices of one and two-electron operators for an arbitrary molecular system, and in particular to deduce the form of the Hamiltonian matrix. The book ends with an account, specially prepared for the English edition, of Hartree-Fock theory in an orthonormal orbital basis, a survey of methods of treating electron correlation and derivations of self consistent field equations for configurations of spin-degenerate states and for a configuration of non-orthogonal orbitals in the method of 'different orbitals for different spins'.

The calculation of a molecular structure involves a number of successive stages: mathematical methods are first of all needed to formulate appropriate equations for the problem; a numerical method must be devised to solve the equations as economically as possible; the solution must be performed to give

numbers; finally the numbers must be interpreted to give insight into the physicochemical problem under study if possible. The laborious and expensive nature of these calculations reflects the fact that none of these stages are trivial; however, Kaplan only considers the first of them. The group theoretical techniques that he describes are immensely powerful, but are sometimes handled in an uncritical way, as in the use of Young tableaux in the description of the Heitler-London calculation for H_2 . Several types of calculation are described, some of which have not yet been implemented in practice, without any discussion of their advantages or disadvantages, consideration of alternative methods, or presentation of numerical results. This lack of critical discussion means that the book is likely to be of most use to the experienced theoretician who wishes to understand how to exploit the techniques in his own work. The novice would find it tough going and would also be put off by the prohibitively high price.

The translation has been carefully done and I only detected a small number of relatively trivial errors. The original Russian text dates from 1969; consequently, the majority of the references are to literature from earlier years though a handful are of more recent origin.
I. P. Grant

Integrated biology

The Peripheral Arterial Chemoreceptors. (Proceedings of an International Workshop.) Edited by M. J. Purves. Pp. xiii+492. (Cambridge University Press: London, June 1975.) £14.00; \$39.50.

THE carotid body seems to have been the subject of more papers (per weight of tissue) than any other vertebrate sense organ; yet it is probably the least understood in terms of its functional morphology. In recent years this situation has been compounded by the publication of conflicting data, which was the main reason for convening a work session at Bristol in 1973. The present book is the outcome of this meeting.

Unfortunately, the editor gives no indication in the Preface or elsewhere in the book as to what the major unresolved problems and areas of contention are. Furthermore, there is no epilogue to indicate whether, or to what degree, the symposium was successful in resolving any of the controversial areas of research. The reader is therefore left with no alternative but to read the entire book to ascertain where the problem areas lie. Not that this is an unrewarding enterprise: all the articles are well written and of a very high standard.

Perhaps the greatest value in this book lies in the discussions at the end of each

paper, for it is among these pages that the reader is made aware not only of the specific problems in carotid-body research, but also of the subjective nature of the so-called exactness of the scientific method. For example, it is apparent that recognition of nerve processes in electron micrographs is achieved at least as much by intuitive as by objective criteria (page 39).

The subject matter ranges from ultra-structural studies, through neuropharmacological, neurophysiological and metabolic aspects of the carotid body sense organ, to circulatory and respiratory chemoreflexes in the whole animal. In general, the presentation is excellent, which is probably why the few minor editorial faults in the book are so irritating. The electron micrographs are displaced from both text and the legends so that the reader has to keep track of three pages simultaneously if he is effectively to follow the anatomical contributions. Some of the electro-neurograms are poorly reproduced and there are a number of mistakes in the reference lists.

Nevertheless, these faults do not detract from the overall excellence of this book, which can be recommended unreservedly not only to mammalian physiologists, but to all who are interested in an integrated approach to problems in biology. M. P. Osborne and P. J. Butler

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Plasma scattering

Plasma Scattering of Electromagnetic Radiation. John Sheffield. Pp. xii+305. (Academic: New York and London, January 1975.) \$29.00; £13.90.

IN this book John Sheffield covers those aspects of the Thomson scattering of laser radiation by a plasma that are relevant to diagnostic applications. The technique, perhaps the most powerful and elegant to have emerged from work on controlled fusion and other laboratory plasmas, presents particular experimental difficulties in view of the very low ratio of scattered to incident light ($<10^{-12}$ – 10^{-14}). In appropriate circumstances, however, it is unique in the direct relationship between observed quantities and basic parameters such as electron velocity distributions and number densities, and in other cases it provides more complex information. Consequently, various aspects have already formed the subject of many reviews.

Problems arise in devoting an entire book to this topic because of the difficult

choice that must be made between presenting a detailed re-exposition of plasma kinetic theory or assuming a background knowledge and adopting an approach more typical, perhaps, of a review article. By and large John Sheffield copes well with this balance. Though highly specialised, the book will clearly be of considerable use to anyone involved in Thomson scattering measurements, as much for physical explanation and theoretical development as for a reference source. Within the confines of the subject the scope is quite wide, with, for example, chapters covering experimental constraints and optical techniques as equally as, say, the theory of scattering from unstable plasmas. The biggest limitation is that the particular theoretical approach chosen is perhaps not the most suited for comparison with scattering processes operating in systems other than plasmas, so that the book is necessarily limited to a very specialised field within plasma physics. Within that constraint, however, I think the book will prove worthwhile and useful. **D.D. Burgess**

Animal development

Practical Studies of Animal Development. By F. S. Billett and A. E. Wild. Pp. xi+251+6 plates. (Chapman and Hall: London, 1975.) £4.80.

Cellular Interactions in Animal Development. By Elizabeth M. Deuchar. Pp. x+298. (Chapman and Hall: London, 1975.) £6.50.

THERE are several good laboratory manuals available for classes in developmental biology. None is, or could be, comprehensive in the sense of dealing with the provenance and manipulation of all the major developmental systems. Each is of most value to teachers not themselves greatly experienced with the material described. *Practical Studies of Animal Development* by Billett and Wild should encourage enterprising teachers of this kind to attempt new laboratory experiments with their classes. The book provides guidance for work with an unusually wide range of animals—echinoids, ascidians, gastropods, annelids, nematodes, insects, teleosts, amphibians, birds and mammals. The exercises are set in the context of their scientific significance, so the book is not just a collection of recipes. Their selection could not suit everyone, but few teachers of developmental biology will fail to find useful matter in this book. In a second edition it might be worth adding a separate section on organ and tissue culture techniques, giving guidance on the nature and source of some of the materials mentioned (for example, MS 222).

We have had for some time in Ebert and Sussex's *Interacting Systems in Development* an admirable introduction to processes which must necessarily play a big part in any epigenetic event. Deuchar, with her *Cellular Interactions in Animal Development*, now offers a more extended, and more detailed, treatment both of the best known and of many less celebrated cases of cell-interactions in animals. There is virtue in reminding students that the more famous examples form but the tip of an iceberg, and I believe that this book will be successful in exciting student interest not just in ideas, but in the systems on which they can be tested. Not many developmentalists, could hope to survey so wide a range of phenomena as Deuchar does, and although she is still only introducing the reader to the problems she deals with, the end result is a very substantial coverage of those parts of developmental biology that are not 'molecular' or 'genetic'.

Her stories are open ended and she indicates areas of doubt with scrupulous care. I could, indeed, have wished that she had given a more forceful statement of her own views in some cases. It wouldn't matter if some later turned out to be wrong. **D. R. Newth**

Allergic diseases

Immunological Aspects of Allergy and Allergic Diseases. Edited by E. Rajka and S. Korossy. Volume 1, Pp. x+456. Volume 2, Pp. xii+457-759. (Plenum: London and New York; Akademiai Kiado: Budapest, 1974.) \$42 each.

THE unprecedented rate of expansion in immunology during the late sixties and the seventies has not been conducive to the compilation of textbooks. Their place has been taken by numerous excellent monographs of an advanced nature while medical students have been catered for by a number of introductory booklets to modern immunology, mostly of exceptionally high standard. The revolutionary new concepts, however, particularly in this field of cellular immunology, have now become consolidated and these two volumes represent one of the first attempts to contain the avalanche of recent information under one roof. The authors are an almost exclusively Hungarian team and have largely succeeded in producing a reasonably up-to-date handbook of immunology for clinicians, with the special interest of the editors-in-chief in atopic allergy largely underplayed.

The first volume deals with the more theoretical basic aspects of modern thought on immune induction and unresponsiveness, whereas the second discusses diagnostic procedures, transplantation and tumour immunology, with two further volumes of a more clinical nature yet to come. Each chapter is generously supplied with references up to and including 1973.

The occasionally quaint phraseology may charm or irritate, but the text is usually clear and controversial matter well argued. In spite of a chapter devoted to terminological aspects, however, confusion sometimes arises from old and new terms being used interchangeably by some authors, and a much needed glossary is lacking.

On page 349 now hardly relevant theories on reagin structure are given a reference to a much later factual paper containing first numerical data on reagin persistence in skin and showing that reagins do not belong to any then known Ig class, but can be assayed by passive sensitisation of chopped lung, data later ignored in their proper context. There is a somewhat irrelevant though well-written chapter on non-Ig serum proteins. In a detailed chapter on the Schwartzman phenomenon, conclusions about its non-immunological nature are reiterated, ignoring recent evidence on massive complement activation as the trigger mechanism. Immunological aspects of nematode infections are not covered in spite of their recent basic interest as regards reagin formation. And although autoimmunity is discussed at length, modern ideas on the bypassing of antigen-specific T-cell cooperation as a fundamental and for the first time integrated mechanism are ignored. The subject index is deplorably incomplete and there is no author index.

Publication of this book coincides with that of an excellent third edition of an established English textbook covering similar ground at roughly half the price for twice the number of pages. **R. Augustin**

obituary

Lancelot Hogben, FRS, populariser of science and author of *Mathematics for the Million*, died on August 22 at Wrexham Hospital in North Wales. He was four months short of his 80th birthday.

Hogben was born in Southsea of aggressively devout Calvinistic parents, educated in the public schools of Portsmouth and was one of the first batch of County Scholarship boys to enter Trinity College, Cambridge, where he studied zoology. The condescension with which he says he was met left deep and ineradicable mental scars. There, he met and was deeply impressed by the philosopher Bertrand Russell. They remained friends until Russell's death. The influence of Russell—in addition to playing a determining part in Hogben's decision to go to gaol as a conscientious objector in the First World War, although possessed of exemption papers—provides the thread of continuity that ran through the astonishingly wide range of subjects to which Hogben made major contributions. He took nothing on trust, and he explored the fundamentals of each discipline before he expounded it. It was this insistence on knowing what he called the "logical credentials" of each subject that made him—like J. B. S. Haldane—one of the very great "popularisers" of science in the tradition of Clerk Maxwell and Michael Faraday.

After graduation from Cambridge he was lecturer in zoology at Imperial College, London (1919–22); assistant director of the Animal Breeding Research Station, Edinburgh (1923); lecturer in experimental physiology, Edinburgh (1923–25); assistant professor of zoology, McGill (1925–27); professor of zoology, Capetown (1927–30); professor of social biology, London

School of Economics (1930–37); Regius Professor of Natural History, Aberdeen (1937–41); Mason Professor of Zoology, Birmingham (1941–47); during much of this time he was seconded as Colonel to the Directorate of Biological Research, later called Directorate of Medical Statistics, at the War Office, to work with his onetime mentor, and friend, the late Professor F. A. E. Crew; Professor of Medical Statistics, Birmingham (1947–61); Honorary Fellow in Linguistics, Birmingham (1961–63); Vice-Chancellor and Principal, University of Guyana (1963–65).

Besides numerous articles in scientific and medical journals (many of fundamental importance) he wrote a wide range of books ranging from textbooks on zoology through the immensely influential *Primers for the Age of Plenty*, of which *Mathematics for the Million* is perhaps the best known, books on language and linguistics, including *Interglossa* (a blueprint for an international language) and *Astroglossa* (for interplanetary communication) to a hilarious and educative tale, all in words of one syllable, called *Whales for the Welsh*. His two Conway Memorial Lectures, "The Retreat from Reason" and "Authoritarianism and Science" deserve re-reading in the present scientific climate. Hogben was a true polymath. He was honoured with the Keith Prize and Gold Medal of the Royal Society of Edinburgh, a Croonian Lectureship.

That is just the bare bones of what Hogben had achieved. What of Hogben the man, funster, punster, controversialist, socialist, enemy, teacher and friend? That Hogben could be awkward, irascible, impossible and sometimes unreasonable seems, from the frequency with which it has been recorded, to be incontrovertible. He did, however, keep these aspects of his

character in the main for those whom he considered were, in virtue of their position, of equal or superior status. He castigated unmercifully what he regarded as meretricious or intellectually dishonest, but he had patience with the obtuse if he thought they showed a genuine desire to comprehend what he had to impart. He was not very good at what he delighted to stigmatise as "oral intercourse", preferring the written word. Possibly this was because his own speech was "lacunic"—terse and pithy but with whole paragraphs elided—and it took some time to become proficient in interpreting this.

He had an enormously stimulating influence on young scientists, both in his own Department and in others. Birmingham University certainly owed much to him. He would also defend the student interest against illiberal oppression by the dinosauric hierarchy. One instance of this happened at a meeting of the Senate at the University of Birmingham towards the end of the war period. There was a move afoot to cut down the autonomy of the Students' Union, ostensibly because a used contraceptive had been in a fire bucket after a dance. One professor, who for years had almost ruled the Senate, said that, speaking as a father, he was horrified to think that he had sent his daughter to a University where such things were used. Hogben replied that, speaking as a father and a grandfather, he would have been horrified if he thought that he had not. Report has it that after a moment's stunned silence the Senate suddenly dissolved into laughter and the whole episode was seen for what it really was. The next day the President of the Students' Union came to thank Lancelot for what he had done, and the long tyranny of the fossilised one was for ever broken.

announcements

Award

Maurice Strong, executive director of the United Nations Environment Programme, is to receive the **Audubon Medal** from the **National Audubon Society** for his role in making the environment an international concern.

Appointments

Professor Michael Sela is to be the next president of the Weizmann Institute of Science.

The University of Adelaide has appointed **Professor Colin J. Driscoll** to the Waite Chair of Agronomy in the Waite Agricultural Research Institute.

Miscellaneous

The second edition of *Collected Tentative Rules and Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature* is now available for distribution. Copies can be obtained from:—The American Society of Biological Chemists, Inc., 9650 Rockville Pike, Bethesda, Maryland 20014.

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